DEDICATED

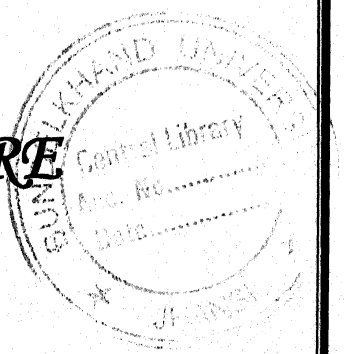
TO

MY LOVING PARENTS

Smt. SHARDA KHARE

&

Shri. RAMESHWAR PRASAD KHARE



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To,

The Vice Chancellor,
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Jhansi

20 September , 2007

Sub: Submission of PhD Thesis
Ref: IGFRI, HEC, No. F. 146/Ph.D./03-Adm dated 8.4.03
BKU/Research/2004/775-75 dated 30.10.04

Sir,

I am forwarding herewith the thesis entitled "Cytogenetical studies on alien introgressions in pearl millet (*Pennisetum glaucum* L.)" by Aarti Khare for the degree of Doctor of Philosophy in Botany, Bundelkhand University, Jhansi. The work has been carried out at Indian Grassland and Fodder Research Institute, Jhansi under the supervision of Dr. P. Kaushal.

Thanking You
Yours faithfully,

K. A. Singh

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CERTIFICATE

It is certified that this thesis entitled "Cytogenetical studies on alien introgressions in pearl millet (*Pennisetum glaucum* L.)" is an original piece of work done by Aarti Khare M. Sc. (Botany) under my supervision and guidance for the degree of Doctor of Philosophy in Botany, Bundelkhand University, Jhansi.

I, further certified that

- It embodies the original work of candidate herself.
- It is up to the required standard both in respect of its contents and literary presentation for being referred to the examiners.
- The candidate has worked under me for the required period at Indian Grassland and Fodder Research Institute, Jhansi.
- The candidate has put in the required attendance in the department during the period.

P. Kaushal
20/9/07

P. Kaushal
Supervisor

DECLARATION

I hereby declare the thesis entitled "Cytogenetical studies on Alien Introgressions in Pearl millet (*Pennisetum glaucum* L.)" being submitted for the degree of Doctorate of Philosophy in Botany, Bundelkhand University, Jhansi (U.P.) is an original piece of research work done by me under the supervision of Dr. Pankaj Kaushal, Senior Scientist, IGFRI, Jhansi.

To the best of my knowledge, any part or whole of this thesis has not been submitted for a degree or any other qualification of any University or examining body in India/elsewhere.

Aarti Khare
20/9/07

AARTI KHARE

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Aarti Khare
20/9/07

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1. INTRODUCTION

Pennisetum L. Rich is one of the largest genera of the tribe Paniceae belonging to the family Gramineae (Poaceae) and with over 140 species distributed throughout the tropics and subtropics of the world (Clayton 1972). The genus includes cultivated species such as pearl millet as well as many other grasses of high fodder value that includes species such as *P. pedicellatum* and *P. purpureum*. The basic chromosome number in the genus varies (x = 5, 7, 8 or 9) as well as the level of ploidy (from 2*n* to 8*n*). Nearly 76% of *Pennisetum* species are polyploids.

Pearl millet (*P. glaucum* (L.) R. Br.) (syn. *P. typhoides* (Burm.) Stapf et Hubb) is an important member of the genus *Pennisetum* which has high importance for both food and fodder. It is the most drought tolerant major cereal, originated in central tropical Africa but cultivated since 1200 BC in India, now widely distributed in the drier tropics. It can produce with as little as 250 mm of annual rainfall. Pearl millet is important because of its ability to produce some grains under environmental conditions too harsh for other coarse grain cereals like sorghum and maize. Some of the desirable features that make pearl millet a model system for genetical and cytogenetical research include its relatively large chromosome size, low chromosome number (2*n*=14), shorter life cycle, protogynous flowering, large number of seeds per plant, asynchronous flowering and high responsiveness to artificial pollination. Important aspects like karyotype, basic chromosome number, cytogenetic stocks, interspecific hybridization, genome relationships, polyploidy and aneuploidy are some of the areas that have received considerable attention. In addition to wide variability within *P. glaucum*, wild species of the genus offers many other desirable traits for pearl millet improvement. Interspecific hybridization and cytogenetic studies are the approches for crop improvement especially in the case where the cultivated genepool lacks the desirable genes.

Pearl millet and napier grass (*P. purpureum* Schum.) are two of the major forage producing *Pennisetum* species in the tropics and subtropics. Pearl millet is known for its high quality forage and significant progress has been made in developing improved cultivars (Hanna *et al.* 1988). Napier grass has been successfully crossed with pearl millet to produce high quality and high yielding perennial interspecific (PMN) forage hybrids (Hanna and Monson 1980, Muldoon and Pearson 1979). Species like *P. polystachyon* and *P. pedicellatum*, that have been overlooked for forage production, are

small seeded apomicts and are not winter hardy but combine excellent dry matter yield with good quality and palatability (Hanna *et al.* 1989). Several other wild *Pennisetum* species that belong to the tertiary gene pool of pearl millet (*P. flaccidum* Griseb., *P. massiacum* Stapf, *P. mezianum* Leeke, and *P. orientale* L.C. Rich) are native to Africa, the Middle East and central and south central Asia are adapted to drier climates. These are valuable forage grasses in the semi-arid regions of the world including the southwestern U.S.

There are many desirable characters that are present in the wild species like stress tolerance, apomixis, pereniality, high tillering etc., if transferred to the cultivated pearl millet, can make it more valuable than ever. The efforts of interspecific hybridization with wild relatives have not yet resulted in commercially viable germplasm except few cases. Success have been achieved in some cases like involving *P. squamulatum*, an aposporous apomict from which attempts were made to transfer gene(s) for apomixis to cultivated pearl millet following backcross method (Ozias Akins *et al.* 2003). The present work includes interspecific hybridization of pearl millet with these above mentioned and other important wild species of the genus for introgression of useful traits to pearl millet. A lot work has been done by various workers for introgression of the desirable traits from wild species to pearl millet, but still major work is required for pearl millet improvement. There have been several advances in the area of pearl millet cytogenetics in the past, and the present study will add more information about the cytogenetic status of pearl millet, wild *Pennisetum* species and their hybrids. Alien introgressions of useful traits from wild species to the cultivated *P. glaucum* is an important aspect which was undertaken in the present study. Wild *Pennisetum* species with potential for pearl millet improvement are given in Table 1.1.

In interspecific hybridizations, one of the major hinderence in getting successful interspecific hybrids is the ploidy barrier of the cultivated and the wild species. Many of the wild species are polyploids while pearl millet is a diploid species ($2n=2x=14$). In the present study, attempts have been made to overcome the ploidy barriers by crossing the species at enhanced ploidy level. Various techniques have been used for the enhancement of ploidy in pearl millet and the wild species. Use of colchicine is one of the methods which have been utilized since decades for induction of polyploidy in the genus.

Another hinderence in interspecific/interploidy hybridization is embryo abortion. The embryo of the hybrid fails to survive due to the disturbance in embryo-endosperm balance number. Various *in vitro* techniques have also been adopted in pearl millet and some wild species to obtain viable interspecific hybrids. Embryo rescue is one of them and will be utilized in the present work where needed.

When a successful interspecific hybrid is produced, the knowledge of its cytogenetic status is must. For that the cytogenetic status of the parents should also be known. Till date, the genomic constitution of various important species in the genus is not known. The genomic constitution of only two species has been assigned viz. *P. glaucum* with AA genome and *P. purpureum* with AA' genome (Krishnaswamy and Raman 1951). The genomic constitution of few other wild species was given by various workers like RR to *P. ramosum* (Marchais and Tostain 1997), SSSSSS to *P. squamulatum* (Dujardin and Hanna 1985) and BB to *P. schweinfurthii* (Vidhya and Khan 2003). Out of all these, partial homology was reported only between the genomes of *P. glaucum* (A) and of *P. purpureum* (A') (Krishnaswamy and Raman 1951). The genomes of other species mentioned above have no or very less affinity with *P. glaucum* genome. In the present project we tried to look for the affinity of some important wild species with *P. glaucum* and the chromosomal behaviour of these species in the interspecific hybrids thus produced and we will also try to look for the genomic constitution of species like *P. orientale* and *P. squamulatum.*

This project mainly concerns with the production of interspecific hybrids between pearl millet (*P. glaucum*) and wild species of the genus *Pennisetum.* Attempts were made for the production of interspecific hybrids and these new hybrids with desirable characters will be selected and could be used for further enhancement of generations for pearl millet improvement.

The present study was undertaken for:-

1. Studies on genomic constitution of various species based on morphological, cytological and biochemical attributes and compatibility with pearl millet at different ploidy levels.
2. Studies on extent of inter and intra- genomic paring in inter and intra ploid crosses.
3. Studies on possibility of introgression of useful traits from wild to cultivated species.

2. REVIEW OF LITERATURE

The present study was undertaken on alien introgressions in pearl millet from the wild *Pennisetum* species by attempting interspecific hybridization. Pearl millet is a very favorable organism for cytogenetic studies and breeding work and has been subjected to intense investigations. The available literature is reviewed under the following headings:

2.1. Taxonomy and botany of the genus *Pennisetum.*

2.2. Gene pools and cytogenetical studies in pearl millet.

2.3. Induction of polyploidy.

2.4. Interspecific hybridization.

2.1. Taxonomy and botany of the genus *Pennisetum.*

Pennisetum has been placed formerly under a variety of genera viz. *Pennicillaria, Holcus, Panicum, Setaria* and *Cenchrus*, before it was settled down in *Pennisetum.* The taxonomic classification presently used for pearl millet is:

Family : *Poaceae*
Subfamily : *Panicoideae*
Tribe : *Paniceae*
Subtribe : *Panicinae*
Section : *Penicillaria*
Genus : *Pennisetum*

The genus is mainly characterized by its inflorescence: a false spike, with spikelet on contracted axes, or spikelets fascicled in false spikes, always surrounded by involucres; the involucres are crowed, with slender, basaily free, glabrous to plumose bristles; the spikelets are sessile or pedicellate, falling with the involucres, only persistent in the cultivated species (Watson and Dallwitz, 1992). The spikelets are lanceolate to oblong, acute to obtuse: the glumes are hyaline or membranous, often unequal, the lower one very small and sometimes absent, the upper one variable, very small to as long as the lemma, with 1-9 nerves. The valve is as long as or shorter than the spikelet, lanceolate to elliptic-oblong, acute, obtuse or truncate, frequently mucronate, rarely 3-lobed. The valvule is narrow, 2-keeled, shorter or as long as the valve, or

suppressed; lodicules minute or absent. The seed is mostly oblong or dorsally compressed, obovoid or subglobose (Stapf and Hubbard, 1934).

As for the sections, most authors accept those recognized by Stapf and Hubbard (1934): Gymnothrix, Eu-Pennisetum, Penicillaria, Heterostachya and Brevivalvula (Table 2.1). Only Brunken (1977) included two species of section Penicillaria in a different section *Pennisetum.* The difference between the sections were often not very strong and can be summarized as follows: in Gymnothrix the spikelets are usually solitary, rarely in clusters of 2-3; the involucres are (sub-) sessile; the bristles are scaberulous, or rarely ciliate; the anthers have glabrous tips, except *P. thunbergii* Kunth. Section Eu-Pennisetum has 1-4 spikelets in each involucre; the spikelets are, if clustered, all alike in shape and usually in sex, or the outer sometimes male, not keeled; the bristles are ciliate, at least the inner ones. Section Penicillaria is further differentiated from other Eu-Pennisetum species by its penicillate anthers, while section Heterostachya has clustered, heteromorphous spikelets, the external male laterally compressed and keeled, and the central hermaphrodite. The last section Brevivalvula is well differentiated from the other sections by the heteromorphous valves, the lower thinly membranous, often three-lobed, the upper shorter, chartaceous, smooth and shining, truncate or very obtuse, ciliolate at the apex; the rachis has decurrent wings below the scars of the fallen involucres (Stapf and Hubbard, 1934). Thirteen species of *Pennisetum* fall into four basic chromosome groups (5, 7, 8 and 9), which in general did not correspond to the morphological sections founded by Stapf (1934). More than one morphological section consisted of taxa with one common basic chromosome number and also one section included taxa from different basic chromosome number groups. The morphological section, Penicillaria was, however, found to correspond with one basic chromosome number group only as shown in the Table 2.1.

The section Gymnothrix comprises 22 species when those that were formerly classified under Beckeropsis are included (Stapf and Hubbard, 1934). The best known species are the very variable *P. macrourum* Trin., *P. ramosum* (Hochst.) Schweinf., and *P. hoheneckeri* Steud. The section *Pennisetum* comprises five species, of which *P. villosum* (R. Br.) Fresen. (feathertop), *P. setaceum* (Forssk.) Chiov. (fountain grass) and *P. clandenstenum* Chiov. (kikuyu grass) are most common. Section Penicillaria comprises seven species, but five of them are probably better classified as cultivars of *P. glaucum* (L.) R. Br. subsp. *glaucum* (pearl millet); the other species is the

well-known fodder grass *P. purpureum* Schum. (napier grass, elephant grass). The section Heterostachya comprises two species: *P. squamulatum* Fresen. and *P. tetrastachyum* K. Schum. (syn. *P. schweinfurthii* Pilg.) and according to Lebrun and Stork (1995) section Brevivalvula comprises three species, *P. pedicellatum* Trin.. *P. polystachyon* (L.) Schult. and *P. hordeoidess* (Lam.) Steud.

Chase (1921) and Brunken (1977) made systematic studies of pearl millet, to clarify the confusion over its name. Nowadays, mostly Chase (1921) is followed who determined *Pennisetum glaucum* (L.) R. Br. as the right name for pearl millet. The names *P. typhoides* (Burm.) Stapf and Hubb. and *P. americanum* (L.) Leeke are still used sometimes.

According to Brunken (1977), *Pennisetum* includes two reproductive isolated species. These are *Pennisetum purpureum* Schumach, (2n=28) perennial species which occurs throughout the wet tropics of the world; and *Pennisetum americanum* (*P. glaucum*) (L.) Leeke, (2n=14) annual species, native to semi arid tropics of Africa and India. The latter contains three morphologically diverse subspecies. The subspecies *americanum* (or *glaucum*) includes a wide array of cultivated pearl millets. Subspecies *monodii* from Sahel in western Africa has been recognized as the wild progenitor of pearl millet. Subspecies *stanostachyum*, which is morphologically intermediate between subspecies *americanum* and *monodii*, is a weedy and mimetic form and often associated with the cultivated pearl millet.

The three subspecies *P. glaucum* ssp. *glaucum*, the cultivated pearl millet, *P. glaucum* ssp. *violaceum* (Lam.) A, Rich., its putative wild ancestor, and *P. glaucum* ssp. *steberianum* (Schlecht.) Stapf and Hubb., comprising all the hybrids formed between the first two subspecies. The first two subspecies remain distinct due to pre-zygotic barriers, resulting in an advantage of self-pollination (Sarr *et al.* 1988, Robert *et al.* 1991) and post-zygotic barriers, resulting in the reduction in viability of hybrid grains (Amoukou and Marchais 1993). *P. violaceum* is grouped in same section as *P. typhoides* i.e. Penicillaria (Stapf and Hubbard 1934) and Brunken (1977). The species belonging to the morphological section Brevivalvula (*P. polystachyon* and *P. pedicellatum*) seem to be more distant from the cultivated species (section Penicillaria) than those in sections Eu-pennisetum (*P. cenchroides*), Heterostyachya (*P. schweinfurthii, P. orientale, P. squamulatum*) and Gymnothrix (*P. hoheneckeri*) on the basis of the pollen pistil interaction studies.

Martel *et al.* (2004) proposed the phylogenetic relationships based on internal transcribed spacer (ITS) sequences to assess evolutionary trends in genome structure within the genus *Pennisetum.* Their results showed that pearl millet and napiergrass form a monophyletic group with a basic chromosome number of x=7 but different chromosome sizes. The ancestral characters of the genus are x=9, small chromosomes, an apomictic mode of reproduction and a perennial life cycle. Species with basic chromosome number of x=5, 7 and 8 appear in the most recent divergent clades, indicates that the genome structure of *Pennisetum* might have evolved towards the reduced chromosome number and an increased chromosome size.

Polyploidy: In the genus *Pennisetum,* nearly three-fourth of the species are polyploids (Jauhar 1981a). *Pennisetum glaucum* is essentially a diploid, though both spontaneous and induced polyploids have been reported. In *Pennisetum* section Brevivalvula, many studies have been undertaken to understand the pairing behavior of the chromosomes in the species. *P. pedicellatum* was reported to be autoploid in origin by Pantulu (1969) and Brunken (1979), while others suggested an auto-alloploid origin (Rangasamy 1972, Sharma *et al.* 1980) or a segmental alloploid origin (Naithani and Sisodia 1966, Sisodia and Raut 1980, Zadoo 1986). An autoploid nature of *P. polystachyon* was reported by Pantulu (1969) and Brunken (1979), while others suggested an autoalloploid origin (Birari 1981, Dujardin and Hanna, 1984), or segmental allotetraploid (Jauhar 1981b). Similarly many species such as *P. subangustum*, *P. atrichum*, and *P. setosum* have been proposed to be auto-allopolyploid in origin (Jauhar 1981b, Brunken, 1979). *P. squamulatum* has been considered to be auto-allopolyploid in origin (Patil *et al.* 1961). This species is believed to be allotetraploid with incomplete homology between the two genomes (Dujardin and Hanna 1983, Jauhar 1981b). Akiyama *et al.* 2006 and Kaushal *et al.* 2007 suggested the species as an octoploid.

2.2. Gene pools and Cytogenetical studies in pearl millet:

Gene pool can be defined as the sum total of all the genes found in the individuals of the population of a particular species. Based on the genetic relationship between species, Harlan and de Wet (1971) developed the concept of gene pools of cultivated crops. In their classification the primary gene pool constitutes species which form fertile hybrids, with regular chromosome pairing and near normal gene segregation, making gene transfer easily attainable. In the secondary gene pool, species hybrids have

a tendency to be sterile, chromosomes pair poorly and sometimes, development is abnormal. Gene transfer in this group is possible with additional efforts. At the level of the tertiary gene pool, access to alien variation is only possible by using special techniques to overcome the barriers isolating the wild species from the cultivated form. The classification of natural variation into distinct gene pools closely follows the concept of biological species. The *Pennisetum* species were divided into gene pools by Harlan (1975) on the basis of their genetic and taxonomic relationships with the cultivated species, *P. glaucum* (comprising of the three subspecies), which is placed in the primary gene pool, with 2n=2x=14. The secondary gene pool includes all biological species that can be crossed with the primary gene pool, but one has to overcome barriers that separate biological species. These barriers include poor chromosome pairing resulting in sterility, lethal or weak hybrids, or poorly adapted hybrid derivatives. It comprises *P. purpureum,* with 2n=4x=28. *Pennisetum glaucum* is a diploid (2n=2x=14) with A- genome while *P. purpureum* is a tetraploid with A'- and B- genomes (Sisodia 1970). The A- and A'- genomes are homeologous, while the B- genome is non homologous to the A- or A'- genome (Jauhar, 1981a). While primary and secondary gene pools incorporate several species based on x=7, the tertiary gene pool consists of perennial, polyploid and apomictic species based on x=9, and several annual species based on x=5 and 8 that can be crossed with pearl millet but the hybrids are lethal, completely sterile, or anomalous. Gene transfer is possible through radical manipulations involving *in vitro* techniques or by using complex hybrid bridges. Occasionally, hybrids among members of tertiary gene pool occur spontaneously (Saideswara Rao *et al.* 1989). *P. schweinfurthii* has been included in tertiary gene pool by Harlan and de Wet (1971), whereas Hanna (1987, 1989) included it in secondary gene pool, as it was possible to cross *P. schweinfurthii* with pearl millet but the hybrid produced was completely sterile. Several *Pennisetum* species in the tertiary gene pool of pearl millet, including *P. flaccidum* Griseb. in Goett., *P. mezianum* Leeke, *P. setaceum* (ornamental species), *P. squamulatum, P. polystachyon, P. pedicellatum* and *P. orientale* L.C. Rich. are of economic importance.

Several analyses have been conducted in the past in order to determine the degree of relationship among the different *Pennisetum* species. They can be divided into analyses that explain species relationships on the basis of qualitative and quantitative phytochemical characters in different taxa, and analyses that explain

patterns on a genetical base. Subba Rao *et al.* (1988), Husein *et al.* (1990) and Saideswara Rao *et al.* (1991) analysed in total 24 different phytochemicals of an almost identical group of 12 *Pennisetum* species. The first authors performed a cluster analysis with the information obtained on 3 aspects, showing neither a strict clustering for species with a same basic chromosome type, nor for species belonging to the same gene pool, nor for a same section. *P. polystachyon,* the only species of section Brevivalvula analyzed, was found to be 60% dissimilar with the other clusters, basically because no protein profile was obtained, and they thus confirmed its belonging to a separate section. The analysis of four isozymes showed that the species relationship among the *Pennisetum* species, originating from all five sections, was often consistent with the available cytogenetic information. In general, there was a closer affinity among species of the **x** = 9 basic number type and among those of the x =7 type (primary and secondary gene pool). *P. mezianum* Leeke, the only species with a x = 8 type, showed closer relationships with the x = 9 types than with both x = 7 types. There was no particular affinity among the x = 9 species of a same section. Most of the species studied, including *P. polystachyon,* showed distinct individual banding patterns. The analysis of 17 free amino acid quantities in leaf extracts revealed no clear information on their phylogenetic affinity, although the profiles in itself were highly species specific. *P. polystachyon* showed the least similarity with *P. orientale* (52.9%) and the highest with *P. squamulatum* (100%). No correlation was found with ploidy level. The results of these phytochemical analyses show basically that the present sections of *Pennisetum* were not based on other than, rather weak, morphological similarities. The clustering of *P. polystachyon* into a separate group in the first analysis was based primarily on the absence of certain leaf proteins. This was questionable because no other species of section Brevivalvula have been analysed, so no generalisations can be made of the section as a whole. In general, most phytochemicals seem to be rather species specific, which make them useful as biochemical markers.

Lagudah and Hanna (1989, 1990) studied enzyme polymorphism of leaves, and later seed proteins and prolamines, in *Pennisetum.* They used 15 wild species and 21 pearl millet inbreds and land races, the accessions originating from different tropical regions. The first analysis showed highly polymorphic zymograms in 4 of the 6 isozymes used. They concluded that the choice of a specific enzyme system might lead to variable deductions on phylogenetic relationships in all three gene pools. For

example, the three species of section Brevivalvula used, *P. polystachyon, P. pedicellatum* and *P. subangustum,* were shown to be closely related on the basis of two enzymes, but highly divergent on the basis of another. In the second analysis prolamine polymorphism was found to be much higher in wild than in cultivated pearl millet, while the prolamins found in *P. purpureum,* of the secondary gene pool, showed a high similarity with this first group. Differences in prolamin composition were revealed among all species of the tertiary gene pool, caused either by the different geographical origin or sometimes to ploidy level. Compared to sections Gymnothrix, Eu-Pennisetum and Heterostachya, the 3 species of section Brevivalvula showed the highest degree of species relatedness, while within this section *P. subangustum* showed more affinity to *P. polystachyon* than to *P. pedicellatum.* Similar results have been obtained by Chowdhury and Smith (1988) based on mitochondrial DNA variation. In this study *P. polystachyon,* and *P. pedicellatum* shared 89% of the total number of restriction fragments. It was suggest that these two species be considered as one species rather than two, which was questionable because not all species of the section have been analyzed. A more general conclusion of these analyses was that although it is difficult to determine the phylogenetic relationships in especially the tertiary gene pool, section Brevivalvula seems to be the only one that is fairly coherent.

Dujardin and Hanna (1989a) studied crossability of diploid and tetraploid *P. glaucum* with eight wild *Pennisetum* species and could obtain hybrids only with three species (*P. setaceum, P. orientale* and *P. squamulatum*). They suggested that species of tertiary gene pool of *Pennisetum* with x=9, cross more readily with pearl millet than those with x=5 (*P. ramosum*) and x=8 (*P. mezianum*). Various morphological, cytological and molecular markers have been utilized to study the species relationship as well as their utilization in pearl millet improvement program. Lagudah and Hanna (1989, 1990) suggested that the distinct isozyme phenotypes of some of the tertiary gene pool species can be explored for possible linkage with desirable traits to be introgressed into pearl millet.

Cytogenetics: Pearl millet is a preferred material for cytological analysis due to its smaller chromosome number (2n=2x=14) and easily stainable relatively larger bivalents. Meiosis has been found to be regular in pearl millet with mostly seven ring-shaped bivalents at diakinesis. Rangaswamy (1935) reported seven ring shaped bivalents having two chiasmata each. Krishnaswamy (1962) also observed mostly ring bivalents at

diakinesis. Jauhar (1981b) found mostly ring bivalents with two chiasmata in different populations, but the nucleolar organizing bivalents was generally rod-shaped with one chiasma. The mean chiasma frequency at metaphase I in this study was 12.1 per cell and 0.9 per paired chromosome in some of the cultivated Indian varieties. Similar results were obtained by Vari (1977). Anaphase separation was regular with 7/7 distribution of chromosomes followed by regular tetrad formation.

Pearl millet being an allogamous species, results in various meiotic irregularities under enforced selfing. The most common irregularity is reduction in chiasma frequency, which was a sort of inbreeding depression for this cytological trait (Pantulu and Manga 1972) and lower mean chiasma frequency than their F_1 hybrids. The reduction in chiasma frequency under stress was also more in inbreds than in hybrids. They also observed heterosis for chiasma frequency which they suggested was partly due to non-allelic gene action. Srivastava and Balyan (1977) also observed heterosis for chiasma frequency and fewer chromosome abnormalities.

The genus *Pennisetum* is cytologically very heterogeneous, chromosome numbers in the species range from $2n$=10 to $2n$ =78. Basic chromosome numbers in this genus are represented by x=5, 7, 8 and 9. Euploids representing from 2x to 8x have been reported while aneuploids are also fairly common (reviewed by Schmelzer 1997). Frequent occurrences of B chromosomes have also been reported in many species (Vari *et al.* 1999).

Some of the desirable features that make pearl millet a model system for genetical and cytogenetical research include its relatively large chromosome size, low chromosome number (2n=14), shorter life cycle, protogynous flowering, large number of seeds per plant, asynchronous flowering and high responsiveness to artificial pollination. Important aspects like karyotype, basic chromosome number, cytogenetic stocks, interspecific hybridization, genome relationships, polyploidy and aneuploidy are some of the areas that have received considerable attention from various workers (reviewed by Minocha 1991, Jauhar and Hanna 1998, Vari *et al.* 1999, Hanna and Gupta 1999). Tostain (1992) studied enzyme diversity in *P. glaucum* subspecies *monodii* in relation to domestication process. This study had shown that wild millet has a genetic diversity not larger than in cultivated millet. The divergence between wild and early-maturing pearl millet was clear. The wild enzymic type for wild millet was characterized by high frequencies of Got Ai, Pgd A3, and Cat A2, and the cultivated type by high frequencies

of Pgi A5 and Pgnz AI. Sympatric wild millet can be distinguished from allopatric, with loci Adh A and Est A more diverse in the former. Sympatric wild millet had a genetic diversity closest to that of cultivated millet. Kaushal and Sidhu (1993) summarized the chemotaxonomic relationship between many *Pennisetum* species. Biochemical studies with respect to isozymes of peroxidase, esterase and acid phosphatase were used to study the relationships between different species of *Pennisetum.* Based on the results, the species were grouped into three categories. The results indicated that the members of same group share greater genomic similarity as compared to inter-group members and the grouping followed earlier classifications by Stapf and Hubbard (1934).

Inghum *et al.* (1993) analyzed the species relationship through occurrence of repetitive DNA sequences. Their results showed that the 140 bp *Kpn*l families of the three related species, *P. purpureum*, *P. squamulatum*, and *P. glaucum* were nearly identical, and thus likely represent a recent divergence from a common progenitor or a common genome. Similarly, genome organization in *Pennisetum* has been analyzed by rDNA and FISH by Martel *et al.* (1996). Using, the 18S-5.8S-25S probe, they observed two sites of distribution in the four species viz *P. glaucum*, *P. violaceum*, *P. mollisimum* (Primary gene pool) and *P. schweinfurthii* (tertiary gene pool), but at different locations. The results showed a chromosomal similarity of rDNA sequence locations within the primary gene pool and were in agreement with the high genetic identity between wild and cultivated forms of pearl millet previously revealed by allozyme studies.

Chabbra *et al.* (2001) reviewed the contribution of isozymes towards various aspects of pearl millet improvement with particular reference to disease resistance, establishment of species relationship, study of genetic and cytoplasmic diversity, and identification of introgressed gene(s).

Diversity in pearl millet and related species has also been studied by Schmelzer and Renno (1997). In their study four euploidy levels (x=9) were assessed by DAPI- flow cytometry for 304 plants of the section Brevivalvula, distributed among five species; *P. hordiodes* (2n=36-54), *P. pedicellatum* (2n=36, 45, 54), *P. polystachyon* (2n=18, 36, 45, 54), *P. setosum* (2n=54), and *P. subangustum* (2n=18,36,54). Genotypic variation expressed by isozyme polymorphism did not show any significant difference between the diploid, sexual populations and the polyploid, apomictic populations of *P. polystachyon* and *P. subangustum.*

Renno *et al.* (2001) studied the haplotype variation of chloroplast DNA in the six morphological taxa (species) of the section Brevivalvula by using RFLP analysis in 54 plants corresponding to 14 elementary taxa, each characterized on the basis of morphology and ploidy level. Two additional species, *P. glaucum* and *P. purpureum,* which belongs to another section of the same genus, were analysed for comparison. Within the Brevivalvula section, plants of *P.setosum,* which were perennial and reproduce vegetatively or by agamospermy, possessed a single specific haplotype. This species differed clearly from the five other morphological species, which were known to be annual., to show either sexual or agamospermic reproduction and which shared most of the 12 other haplotypes observed in the section, suggesting the occurrence of multiple hybridization events between the taxa. Chloroplast DNA variation was highly geographically structured, suggesting low seed dispersal between sites, whereas the substantial haplotype diversity observed in the sites may indicate that agamic reproduction was responsible for the maintenance of distinct genetically isolated clones. Haplotype classification using Wagner's parsimony suggested the occurrence of bidirectional gene flow between the diploids and the polyploids.

Martel *et al.* (2004) reported the phylogeny and chromosomal evolution within *Pennisetum.* The tertiary gene pool species, genetically isolated from domesticated pearl millet, were included in the two basal divergent groups. The primary and secondary gene pool species form a monophyletic clade, which appeared as a subgroup of one of the tertiary gene pool groups, indicating a more recent divergence. Highly supported phylogenetic proximity was observed between morphologically related species, i.e. *P. ramosum* and *P. mezianum* (section Gymnothrix), *P. schweinfurthii* and *P. squamulatum* (section Heterostachya), *P. polystachyon* and *P. pedicellatum* (section Brevivalvula), and *P. hoheneckeri* and *P. alopecuroides* (section Gymnothrix). The phylogenetic analysis suggested that the chromosome complement in *Pennisetum* had evolved from a basic chromosome number x=9 with short length. This conclusion contradicts the earlier assumption of an ancestral basic number x=5 based on chromosome paring in haploids and interspecific hybrids (Jauhar 1970b, 1981b, Rao *et al.* 1989), but was in agreement with the chromosome evolutionary trend generally observed in grasses.

2.3. Induction of polyploidy:

Several methods have been used to induce polyploidy in plants. Some of them are heat and cold treatment, gamma rays and chemicals like acenapthene, 8-hydroxy quinoline and nitrous oxide. The most effective method of chromosome doubling is the treatment of seeds, seedlings or shoot tips with colchicine. The method of inducing polyploidy in plants was developed independently by Blakeslee and Nebel in 1937. Following them a number of workers obtained polyploids in pearl millet as well as in other crops experimentally. Colchicine disturbs the spindle formation during cell division thus causing chromosome doubling (Blakeslee 1941).

Khushk *et al.* (1987) treated seeds and seedlings of *Trifolium alexandrinum* L. by colchicine solution, and obtained best results from 0.04 % concentration for 24 hours in seed treatment and 4 hours at the same concentration from the seedlings. The tetraploids showed the gigantism for number of morphological traits of plants. Those performed by seed treatments performed better than seedling treatment. An appreciable increase in pollen grain size was noted in the tetraploids.

Polyploidization and its effects were studied in diverse crops including *E. grandiflorum* (blue poppy cultivar; Griesbach and Bhat 1990), maize (Balan *et al.* 1990) by treated clones, varieties and hybrids with gamma and U. V. radiations and with chemical mutagens (nitroso compounds and colchicine), *Vaccinium elliottii* Champ plant (2n=2x=24) (Dewikat and Lyrene 1991), *Solanum chacoense* (Freck 1991) by using dimethyl sulfoxide (DMSO) and plant growth regulators gibberllic acid (GA3) and benzyl aminopurine (BAP), lucerne, red clover and cock foot (Dactlysis glomerata) (Smith *et al.* 1991), *Capsicum annum* L. Var. New Red Hot (Khan and Siddiqui 1992), black pepper (*Piper nigram* L.) (Nair and Ravindran 1992), *Phlox drummondii* (Verma *et al.* 1993), *Cajans cajan* cv. Prabhat and *Vigna unguiculata* ssp. *unguiculata* cv. GC 82-7 (Patel and Verma 1999), Various other workers obtained induced autotetraploids in plant species like rye (Aslim 1994), Solonum melongena cv. Giwa (Sangowawa 1994), V. narborensis (Singh and Singh 1999), Trichosanthes dioica (Hazra and Ghosh 2001), *Trifolium alexandrinum* (Roy *et al.* 1998).

F_1 hybrid plants (*C. platycarpus* x *C. cajan*) obtained by rescuing aborting hybrid embryos *in vitro* (Mallikarjuna 1998). Apical buds of these F_1 hybrids ($2n = 22$) were treated with an aqueous solution of 0.05% colchicine with 10% Tween-20 using a soaked cotton swab placed on the apical buds. After three days of colchicine treatment,

apical buds were washed with water and allowed to grow. All the auxiliary buds and branches were excised. Hybrids were selfed to obtain tetraploid F_2 ($2n$ = 44) progeny. The F_1 hybrids of Pigeonpea (*Cajanus cajan* (L.) Millspaugh) and *Cajanus platycarpus* with 2n=22 were treated with colchicine to obtain tetraploid hybrids with 2n=44 chromosome number, that were selfed to obtain F_2, F_3 and F_4 progenies.

Induced tetraploidy in pearl millet has been utilized to attempt interspecific and intergeneric crosses, for studying genomic relationships and for transferring genetic material from species with higher ploidy status. The occurrence of tetraploids in pearl millet was first reported by Powell and Burton (1968) in plants arising form poly-embryonic caryopsis. These tetraploids were not distinguishable from diploids by their morphological features at vegetative stage but could be identified by their tufted inflorescence and poor pollen shedding. Koduru and Krishna Rao (1978) reported four autotetraploids in the progeny chromosome pairing typical of an autotetraploid, all except one showed chromosome pairing typical of an autotetraploid, while the fourth had a high frequency of univalents that might have arisen due to desynapsis.

Synthesis of tetraploids in pearl millet by colchicine treatment was first reported by Krishnaswamy *et al.* (1950). They induced tetraploidy by administering aqueous solution of colchicine in the shoots of young seedlings. Later on, several workers have induced tetraploidy by colchicine treatments (Gill *et al.* 1969, 1970, Jauhar 1970a, Jha and Sinha 1986).

Gill *et al.* (1969, 1970) characterized the colchicine induced tetraploid (4x=28) plants as having lower seed set, larger stomata and pollen grains and multivalent chromosome associations.

Hanna *et al.* (1976) conducted studies on relationship of autotetraploids in *P. glaucum* to polyembryony, their morphology, reproductive behavior, and cytology. Earlier workers reported poor pollen shed, reduced pollen stainability, pollen size variability, and highly reduced seed set in induced tetraploids. In contrast, Kaushal *et al.* (1999) reported highly fertile and high seed setting induced autotetraploid in pearl millet. These tetraploids were later utilized to produce interspecific hybrids between pearl millet and polyploid *P. squamulatum* (Kaushal *et al.* 2003a).

Meiosis in tetraploids was studied by various workers and large variation was found in the frequency of bivalents and quadrivalents. The C_1 generation

autotetraploid studied by Raman *et al.* (1962) showed a mean individual bivalent frequency of 2.6 per cell, with mean pollen sterility of 16.5%. Hanna *et al.* (1976) observed a mean chromosome association of 1.4_{IV} $+6.38_{III}$ $+8.97_{II}$ and 2.64_{I} in the tetraploids of inbred Tift 239. Gill *et al.* (1969) and Jauhar (1970a) reported a mean quadrivalent frequency of 3.01 and 4.31, respectively, for the colchicine induced tetraploids. Minocha *et al.* (1972) compared the multivalent frequency in tetraploids of an open pollinated variety T 55 and its inbred BIL 4 and found that the inbred line had a mean of 3.01_{IV} $+_{III}$ per cell while the open pollinated had only 2.54_{IV} + $_{III}$ per cell, indicating that the degree of heterozygosity in the parents could influence the chromosome associations in tetraploids. Similar observations were made in inbred lines of tetraploid rye (Hossain and More 1975). Singh *et al.* (1977) observed only bivalent association in gamma-ray induced tetraploids.

Gill *et al.* (1969) and Jauhar (1970a) studied the chromosome pairing in the raw and advanced generations of tetraploid population and found that cytological diploidization (gradual shift from multivalents to bivalents) occurred in colchicine induced tetraploids. According to Jauhar (1981a) it may be due to natural selection for genes which condition regular meiosis with bivalent formation. Another interesting observation made in pearl millet tetraploids was the reversion of tetraploidy into diploidy in subsequent generations. Diploids were recovered in C_2 generation (Raman *et al.* 1962), C_3 generation (Subba Rao 1978), C_3 and C_4 generations (Gill *et al.* 1969), and upto C_6 generation (Jauhar 1970a, Jauhar *et al.* 1976).

The fertility in tetraploids was found to be much lower than that in the parental diploids, and it increased with advancement of generations (Gill *et al.* 1969, Jauhar 1970a). Hanna *et al.* (1976) argued that one of the factors responsible for poor seed set in tetraploids could be the production of unbalanced gametes due to multivalent formation. While Jauhar (1970a) inferred that meiotic abnormalities coupled with genetic factors were jointly responsible for sterility in tetraploids. It was suggested that fertility in autotetraploids has a genetic basis and if selection is practiced, fertility could be improved in subsequent generations. They observed that increased fertility in selected populations was associated with increased chiasma frequency, increased quadrivalent frequency and regular distribution of chromosomes at anaphase.

Gill *et al.* (1969) reported an increase in the frequency of bivalents (7.87 per cell in C_0 and 10.81 per cell in C_5 generation) in the autotetraploids that showed a

high degree of seed sterility. In general, an improvement in the seed set in the advanced generations has been observed, and it has been shown to be possible to obtain lines through selection which has either significantly higher or lower fertility than the base population. The autotetraploids do not show much promise as a forage crop either (Minocha *et al.* 1972).

Intercrossing two semi-sterile tetraploid pearl millet inbred lines Tift 23BE and Tift 239DB, resulted in tetraploid F_1 hybrids and F_2 progeny which had higher fertility than the parental inbred lines. Each parental line set less than 1% and 3% selfed and open pollinated seeds, respectively. Seed germination was usually less than 17%. The F_1 hybrids between the two inbreds set upto 61% seed while upto 40% of the seeds from hybrids germinated. Fertility improvement was reflected by an increase in pollen shed, seed set, seed quality, and seed germination (Dujardin and Hanna 1989c).

The induced tetraploid of *P. schweinfurthii* was obtained by giving colchicine treatment was similar in appearance with diploid but had decreased size of pollen grains, low seed set, reduced seed size, and reduced pollen and seed fertility (Khare *et al.* 2004).

2.4. Interspecific hybridization:

Interspecific and intergeneric hybridization are the approaches to introduce genes for desirable characters into economically important cultivated crops, which are absent in cultivated gene pool. Gene pools can be widened through hybridization of crop cultivars with wild species and weedy races, as well as through interspecific crosses between diverse germplasm groups. Experimental evidence indicates that wide crosses can be used for improving many traits, including yield. Various techniques were utilized, where direct hybridization was not possible, for successful interspecific hybridization like bridging species, radiation induced translocations or by induced homeologous recombination were used. A few notable examples of the successful transfer of alien genes for improvement of cultivated cereals are discussed here.

Stem rust resistance was transferred to bread wheat from *Triticum durum, T. dicoccum* and *T. monococcum* (Hayes *et al.* 1920, McFadden 1930 and McIntosh *et al.* 1984), and from *Agropyron elongatum* (Knott 1961, 1971), Cytoplasmic male sterility from *T. timopheevi* (Wilson and Ross 1962), Leaf rust resistance from

Agropyron elongatum (Sears 1973) and high protein from *T. dicoccoides* (Kushnir and Halloran 1984). The characters transferred to cultivated oat (*Avena sativa*) through interspecific hybridization include crown rust resistance from *A. strigosa* (Browning and Frey 1969), increased biomass and grain yield from *A. sterilis* (Frey 1983) and mildew resistance from *A. prostrata* (Griffiths and Thomas 1983). In rice, Grassy stunt virus resistance was transferred from *Oryza nivara* (Khush 1977) and cytoplasmic male sterility from *O. perennis* (Lin and Yuan 1980). Genes for resistance to bacterial leaf blight (Xa21) from *O. longistaminata* were transferred to the cultivated rice (Khush *et al.* 1990). Northern corn leaf blight resistance was transferred to corn from *Tripsacum floridanum* (Simon and Hooker 1976) and resistance to northern corn leaf blight, anthracnose and common rust from *T. dactyloides* (Bergquist 1977). In barley, genes for various characters such as powdery mildew resistance (Rudorf and Wienhues 1951), cytoplasmic male sterility (Scooler 1967) and increased biomass and grain yield were transferred from *Hordeum spontaneum* (Frey 1983).

QTLs for higher yield potential were discovered from exotic germplasm and tagged with molecular markers. The tagged QTLs were transferred to elite lines in tomato and rice (Tanksley and McCouch 1997). A cross involving *O. rufipogon*, a wild species of rice, and an elite hybrid variety of cultivated rice was studied. In a BC_2 testcross population, transgressive segregants outperforming the original hybrid variety were observed (Xiao *et al.* 1996).

Desirable genes for pearl millet improvement such as stress tolerance, resistance against ergot higher biomass yield, better nutrition and apomixis has been reported in many wild species (Kannaiyan *et al.* 1972, Hanna 1987, Hanna and Bashaw 1987, Wilson and Hanna 1992, Singh and Navi 2000). Interspecific hybridization involving such species has been attempted. Hanna *et al.* (1985) reported the transfer of alien genes for rust resistance to pearl millet by recombination due to genome homology between pearl millet and a wild subspecies, *P. violaceum* (*P. glaucum* ssp. *monodii*). *P. violacuem* has also been used as a source for cytoplasmic male sterility (Marchais and Pernes 1985, Hanna 1989) as well as thermosensitive genic male sterility in *P. glaucum* (Kaushal *et al.* 2004).

2.4.1. Pre-fertilization barriers: In family Gramineae, incompatibility restricted the recovery of first generation hybrids is due to pre-fertilization barriers operating between pollen reception and the entry of the pollen tube into the embryo sac. Heslop-Harrison

(1982) mentioned the loss of tube orientation with random growth and failure of the tubes to locate the micropyle as one of the causes of pollen tube rejection causing cross-incompatibility in grasses.

The pre-fertilization barriers resulting interspecific incompatibility, can be circumvented by the production of autotetraploids of the cultivated species prior to making the cross with the wild species. This approach was employed in producing several fertile hybrids between cultivated pearl millet and *P. squamulatum* but was unsuccessful with other species such as *P. mezianum* and *P. macrourum* (Dujardin and Hanna 1983, 1989a).

According to Mohindra and Minocha (1991), the interspecific incompabilities in the genus *Pennisetum* were expressed with impediment to pollen tube growth at different regions of pistil. The results are described later in this section. Chaix and Marchais (1996) and Marchais and Tostain (1997) were with same opinion that interspecific pollen-pistil incompatibility was independent of the ploidy level of the parents.

Chaix and Marchais (1996) showed that prezygotic incompatibility barriers erected by pearl millet gynoecia against foreign pollens were stronger with sympatric pollinator species than with allopatric ones. Of the five *Pennisetum* species tested as male partners, the three highly compatible ones (*P. ramosum, P. schweinfurthii* and *P. squamulatum*) were allopatric to pearl millet and the two totally incompatible ones (*P. pedicellatum* and *P. polystachyon*) were sympatric to pearl millet. Sorghum provides another example of incompatibility barrier existing between pearl millet pistils and sympatric foreign pollens. Most of sorghum pollen tubes were stopped in pearl millet styles and did not reduce significantly subsequent fertilization of pearl millet by its own pollen (Reger and Sprague 1982).

To identify the stages of pre-fertilization impediments to interspecific hybridization, the behavior of the pollen tubes of four wild *Pennisetum* species was investigated in the stigmatic tract of *P. glaucum* by Kaushal and Sidhu (2000). The pollen tube germination and growth was normal in *P. glaucum* x *P. violaceum* as it was a compatible cross while the delayed and restricted growth of pollen tubes indicated that the barrier operated in the styllar hairless region for the crosses with *P. squamulatum* and *P. orientale,* and at the ovarian level for the *P. glaucum* x *P. setaceum* cross.

2.4.2. Embryo rescue: The failure of endosperm to develop, leading to the death of potentially viable hybrid embryos, can be overcome by culturing the developing embryo on artificial medium. From the inception of zygote formation through to germinate, the occurrence of post-fertilization disorders constitutes a major hurdle to stable hybrid embryo development in interspecific crosses. The application of tissue culture technique, particularly in the area of embryo rescue, has had a major impact on the maintenance and development of hybrid embryos from interspecific crosses. The dynamics of nutritional requirements, osmotic adjustments, and physical conditions such as temperature, light, and gaseous environment must be optimized for a successful embryo rescue. Since the first demonstration by Laibach (1925, 1929) that embryos from nonviable seeds of *Linum perenne* x *L. austriacum* hybrid could be cultured on nutrient medium and raised to maturity, the technique has been routinely used for producing hybrids. Embryo rescue has been successfully used to produce hybrids involving interspecific and intergeneric crosses in cereals. Notable examples were wheat x barley, wheat x rye, and maize x *Tripsacum* crosses.

Various culture media and techniques have been developed for several crop species (reviewed by Williams *et al.* 1987). The excision stage of the embryo for *in vitro* culture either before or after the transition from the globular to the heart stage of development is an important factor in successful embryo rescue. There are few reports of successful cultures from the globular stage because induction of bipolarity at the heart stage is thought to be associated with critical growth regulators and the asymmetry of the embryo's location within the ovule. In wide crosses, where few embryos are produced, the efficiency of recovery of viable hybrid plants may be enhanced by callus induction from the embryo and subsequent regeneration of plantlets. These procedures are directed towards obtaining more efficient survival of embryos in situations where very immature embryos are to be cultured. Embryo culture was successfully used to produce hybrids of wheat with *Agropyron ciliare, Ag. Trachycaulum, Ag. Yezoense,* and *Ag. Scirpeum* (Sharma and Gill 1983).

Various *in vitro* techniques have also been adopted in pearl millet and some wild species of the genus *Pennisetum*. The earliest attempts on *in vitro* culture of pearl millet can be traced back to Narayanaswamy (1959). He reported outgrowth of the scutellum of the excised embryos under culture conditions and formation of non-morphogenic callus from it. Interspecific hybrids were produced between *P. glaucum*

and *P. schweinfurthii*, *P. orientale* and *P. mezianum* by embryo rescue. The hybrid produced between *P. glaucum* and *P. mezianum* was the first report and was possible only by using embryo rescue technique (Nagesh and Subrahmanyam 1996). According to Marchais and Tostain (1997), ploidy levels of mating partners does not influence pistil-pollen compatibility, but play a major role in post-zygotic abortion. The author further described that much of the reproductive isolation can be bypassed with the help of embryo rescue and chromosome doubling by colchicine. Successful embryo rescue technique in interspecific hybridization in *Pennisetum* was used by various workers (Nagesh and Subrahmanyam 1996, Marchais and Tostain 1997, Kaushal *et al.* 2004)

Embryo rescue technique in pearl millet has been advanced for "embryo cloning", whereby 25-30 plantlets could be obtained from single rescued embryo (Kaushal *et al.* 2003b). This technique has great potential in obtaining appreciable number of hybrid plantlets even when the initial rescued embryos could be less in number.

Hybrids were obtained between pearl millet and its wild relatives *P. ramosum, P. schweinfurthii, P. squamulatum* by embryo rescue (Marchais and Tostain 1997), which are discussed later in this review.

2.4.3. Interspecific hybridization in cereals: In recent years, experimental hybridization has been effected between taxonomically distant taxa. In Gramineae family, hybrids have been reported between *Oryza sativa* x *Pennisetum spp.* (*P. orientale* 2n=18; Wu and Tsai, 1963), *Saccharum officinarum* x *Sorghum bicolor* (de Wet *et al.* 1972), *O. sativa* x *S. bicolor* (Zu *et al.* 1985), *Triticum aestivum* x *Agropyron sensu lato,* and many others.

Several alien genes for disease resistance were transferred from wild species into the cultivated varieties of wheat, oats, corn and rice. As an example, genes for stem rust resistance were transferred from wild species like *Triticum durum, T. dicoccum* etc. to *T. aestivum* (Hayes *et al.* 1920, McFadden 1930), a gene for grassy stunt resistance from *Oryza nivara* to cultivated rice was transferred through backcrossing (Khush 1977). Genes for increased biomass and grain yield were transferred from wild species into cultivated varieties of oats, sorghum, and pearl millet (Frey 1983).

A classic example of successful intergeneric hybrid was *Triticale*. Triticale is the first man made crop derived by crossing wheat (*Triticum*) and rye (*Secale*). Depending upon whether tetraploid (2n=4x=28) or hexaploid (2n=6x=42) wheat was utilized for the synthesis, one would get hexaploid *Triticale* (2n=6x=42) or octoploid *Triticale* (2n=8x=56). In each case only diploid rye (2n=2x=14) was used. Work done on this crop has been reviewed in Muntzing (1939) Gupta and Priyadarshan (1982).

The main problem associated with the use of wide crosses to introduce new genes in cereals is the transfer of undesirable genes. In wheat varieties carrying a rye/wheat translocation (IR/IB), for example, the genes for resistance to stem and leaf rust and powdery mildew were introduced, but new problems arise in the bread making quality of the flour prepared from the respective wheat varieties (Baum and Appels 1991, Mettin *et al.* 1973). Techniques to reduce the amount of transferred alien chromatin carrying undesirable genes include irradiation or mutations that allow recombination between the alien chromatin and the wheat chromosomes. The use of somaclonal variation in tissue culture for interchromosomal transfer of smaller segments of alien chromatin was reported by Larkin and Scowcroft (1981).

Interspecific hybridization has been used not only to transfer valuable genes from one crop to another but also, in conventional breeding programs, for the production of haploids. Kasha and Kao (1970) developed a haploid production method for barley breeding by chromosome elimination in crosses of *Hordeum vulgare* with *H. bulbosum*. Other species such as maize (Laurie 1989, Laurie and Bennett 1988a), pearl millet (Laurie 1989), and sorghum (Laurie and Bennett 1988b) have also proven successful in crossing to wheat, for the purpose of producing haploid wheat.

2.4.4. Interspecific hybridization in *Pennisetum*: Attempts have been made to transfer alien genes from primary, secondary as well as tertiary gene pools for pearl millet improvement. The wild gene pool contain important genes that might be transferred to cultivated pearl millet and utilized in creating useful genotypes (Harlan 1975, Hanna 1987). Cytogenetical studies involving interspecific crosses in pearl millet are limited. Crossability status of wild *Pennisetum* species with pearl millet has been reviewed by Marchais and Tostain (1997) and Dujardin and Hanna (1989a). Incompatibility barriers between pearl millet and wild *Pennisetum* species have been reported by Mohindra and

Minocha (1991), Chaix and Marchais (1996), Marchais and Tostain (1997) and Kaushal and Sidhu (2000). Kaushal and Sidhu (2000) observed that pollen germination was normal in crosses with *P. violaceum*, *P. squamulatum* and *P. orientale* and slightly inhibited in a cross with *P. setaceum.*

Desirable genes for pearl millet improvement such as stress tolerance, higher biomass yield, better nutrition and apomixis has been reported in many wild species (Hanna 1987, Wilson and Hanna 1992, Singh and Navi 2000). Napier grass has been successfully crossed with pearl millet to produce high quality and high yielding perennial interspecific (PMN) forage hybrids (Hanna and Monson 1980, Muldoon and Pearson 1979).

A number of *Pennisetum* species in the tertiary gene pool appear to have desirable forage characteristics. Some of these include *P. pedicellatum* Trin. (Tyagi and Singh 1986, Singh *et al.* 1988, Hanna *et al.* 1989, Mishra and Katiyar 1990) *P. orientale* L. C. Rich. and *P. flaccidum* Griseb (Burns *et al.* 1978, Hanna *et al.* 1989), *P. polystachyon* (L.) Schult., *P. squamulatum* Fresen. and pearl millet-napier grass-*P. squamulatum* intercrosses (Hanna *et al.* 1989).

Species in the tertiary gene pool are generally polyploid and apomictic (Jauhar 1981b). Most apomictic species also show a low level of sexual reproduction. The sexual reproduction can be used to produce hybrids with new gene combinations. Superior heterozygous apomictic clones selected from wild populations or developed through hybridization can be propagated by seed. Hanna (1995) discussed various approches for using apomixis in cultivar development.

Using pearl millet as a pollen parent in crosses with barley, Zenkteler and Nitzsche (1984) obtained globular embryos. In crosses between hexaploid wheat cv. Chinese Spring and the pearl millet genotype Tift 23 BE, Laurie (1989) observed fertilization in 28.6% of the 220 florets pollinated. Chromosome counts in zygotes confirmed the hybrid origin of the embryos; three embryos had the expected 21 wheat and 7 pearl millet chromosomes and a fourth had 21 wheat and 14 pearl millet chromosomes. However, the hybrid embryos were cytologically unstable and probably lost all of the pearl millet chromosomes in the first four cell division cycles. The elimination of pearl millet chromosomes at an early stage limited the chances of gene transfer from pearl millet into wheat.

Jauhar (1981b) and Patil and Singh (1980) summarized studies on various intergeneric hybrids involving *Pennisetum* species. Most intergeneric hybrids were weak, and/or more information was needed to establish their usefulness. Hussey *et al.* (1993) reported on a 2n+n *Cenchrus ciliaris* x *P. orientale* intergeneric hybrid that had excellent forage potential.

In crosses between five cultivars of oat with pearl millet (as pollinator), Matzk (1996) obtained a hybrid frequency of 9.8%. However, the pearl millet chromosomes were lost during embryo or plant development. In one hybrid, 5 pearl millet chromosomes were retained with 21 of oat. Hybrids like this could offer an opportunity for transfer of pearl millet genes into oat or vice versa. Such hybrids could also help produce alien addition or substitution lines in the two crop plants. Bridging species can be used to increase success of interspecific/intergeneric crosses.

According to Chaix and Marchais (1996), *Cenchrus ciliaris, Panicum maximum and Zea mays* showed higher compatibility levels with pearl millet than *P. pedicellatum* and *P. polystachyon.* Taxonomic distance was therefore not strictly linked to incompatibility. *P. ramosum, P. schweinfurthii* and *P. squamulatum,* genetically more distant from pearl millet than were *P. pedicellatum* and *P. polystachyon* (Chowdhury and Smith 1988) but highly compatible with pearl millet, do not support the hypothesis that incompatibility is correlated with genetic distance. It was observed that pre-zygotic incompatibility barriers erected by pearl millet gynoecia against foreign pollens were stronger with sympatric pollinator species than with allopatric ones. Indeed, of the five *Pennisetum* species tested as male partners, the three highly compatible ones (*P. ramosum, P. schweinfurthii* and *P. polystachyon)* were sympatric to pearl millet.

2.4.4.1. *P. glaucum* and species of primary gene pool: Interspecific hybridization involving species with useful genes had been attempted. Hanna *et al.* (1985) reported the transfer of alien genes for rust resistance to pearl millet by recombination due to genome homology between pearl millet and a wild subspecies, *P. violaceum* (*P. glaucum* ssp. *monodii*). *P. violaceum* has also been used as a source for cytoplasmic male sterility. In addition to A_1, A_2 and A_3 CMS sources (Burton and Athwal 1967) used in hybrid breeding, two other sources (A_4, A_v) derived from different accessions of *P. violaceum* (Marchais and Pernes 1985, Hanna 1989), have been reported. *P. violaceum*, being sexually compatible with pearl millet, is an important source of stable male sterility inducing cytoplasm (Rai *et al.* 1996). Chhabra *et al.* (1997) studied the influence of a

range of cytoplasm on microsporogenesis and anther development in pearl millet using six iso-nuclear A-lines having five cytoplasms (A_1, A_2, A_3, A_4 and A_v) and the nuclear genome of maintainer 81B. They concluded that each cytoplasm had its unique influence on microsporogenesis and anther development, as evidenced by different developmental paths followed for pollen abortion. The cause of pollen abortion differed from line to line, from floret to floret within a spikelet, from anther to anther within a floret, and in some cases even from locule to locule within an anther. Events that led to male sterility included anomalies in tapetum and callose behavior, persistence of tapetum, endothicium thickness, and other unknown causes. Kaushal *et al.* (2004) reported thermo sensitive genic male sterility (TGMS) in *P. glaucum* that was recovered from embryo culture of a cross between *P. glaucum* and *P. violaceum.* The behavior of male sterility was temperature-dependent, as the development of pollen mother cells to pollen grains was found to be dependent on temperature. The plant showed sterility at lower temperatures while it was fertile at higher temperatures during seasonal variations. This thermosensitive male sterility trait was found to be genetically controlled.

Liu *et al.* (1996) studied the effects of homeology and sex on recombination frequency in crosses between cultivated pearl millet, *Pennisetum glaucum,* and two wild relatives, *P. violaceum* and *P. mollissimum.* For the two wild x cultivated crosses, reciprocal three-way crosses were made between the F_1 hybrid and an inbred line (Tift 23DB1). The three-way cross populations were mapped to produce a female map of each wide cross (where the F_1 was the female) and a male map (where the F_1 was the male). Total genetic map lengths of the two inter-subspecies crosses were broadly similar and around 85% of a comparable intervarietal map was constructed. Comparison of the recovered recombinants from male and female meiocytes showed an overall trend for the genetic maps to be longer in the male (10%) in both inter-subspecific crosses; however, analysis of individual linkage intervals showed no significant differences. Gametophytic selection was prevalent, and sometimes extreme, for example 12:1 in favour of 'wild' alleles in the *P. glaucum* x *P. mollissimum* male recombinant population. One of the loci which determines panicle type in cultivated pearl millet and wild relatives, H, was mapped 9 cM from Xpsm812 on linkage group 7 in the *P. violaceum* cross.

Poncet *et al.* (1998) assessed the inheritance of domestication traits distinguishing pearl millet (*Pennisetum glaucum*) from its wild relative *P. mollissimum*

was assessed in F_2 progenies derived from a cross between a typical landrace of pearl millet and a wild ecotype. Despite a high level of recombination between the two genomes, the existence of preferential associations between some characters was demonstrated, leading, in particular, to cultivated-like phenotypes. Traits determining spikelet structure showed simple Mendelian inheritance. The genes encoding these traits mapped in a linkage group where quantitative trait loci for spike size and tillering habit were found. This linkage group could correspond to one of the two chromosome segments that have been involved in the variation for spikelet structure in progenies from several cultivated×wild crosses.

2.4.4.2. Interspecific Hybridization with the species of secondary gene pool

P. glaucum* x *P. purpureum

P. purpureum (napier grass or elephant grass) with 2n=4x=28, is another important wild species in the genus and is used for both forage and biomass production. It contains many desirable genes like disease resistance, perenniality, high dry matter yield and high quality forage for pearl millet improvement. The two species *P. glaucum* (2n=2x=14) and *P. purpureum* cross easily.

The earliest report on interspecific hybrid between *P. glaucum* (2n=2x=14) and *P. purpureum* (2n=4x=28) was by Burton (1944), followed by Krishnaswamy *et al.* (1950), Gildenhuys (1950), Krishnaswamy (1951), Gildenhuys and Brix (1958, 1964), Khan and Rehman (1963), Pritchand (1971) and Dhanapala *et al.* (1972). Later on, interspecific hybridization involving *P. purpureum* (napier grass) has been extensively attempted for improvement of forage characteristics in pearl millet (Patil and Jadhav 1992, Spitaleri *et al.* 1994, Sukanya 1997, Liang and Liang 1999) Genes controlling earliness, long inflorescence, leaf size, and male fertility restoration for improving pearl millet have also been transferred from *P. purpureum* (Hanna 1983), in addition to produce high quality and high yielding perennial interspecific (PMN) forage hybrids (Muldoon and Pearson 1979, Hanna and Monson 1980;).

Germplasm of the A' genome of *P. purpureum* was transferred to cultivated pearl millet by pollinating cytoplasmic nuclear male sterile pearl millet with fertile allohexaploid *P. glaucum* x *P. purpureum* hybrids (Khan and Rahman 1963, Hanna 1979, 1990). The F_1 hybrid between *P. glaucum* and *P. purpureum* was a triploid (2n=3x=21) with 7 chromosomes from *P. glaucum* and 14 from *P. purpureum* and was sterile with genome AA'B. Krishnaswamy (1951) and Krishnaswamy and Raman (1953,

1954) studied the chromosome pairing in the hybrid and its amphidiploid. They observed high frequency of PMCs with 7_{II} $+7_{I}$ association and concluded that one of the genomes of *P. purpureum* is homologous with *P. glaucum* genome and suggested genomic formulae of AA for *P. glaucum* and AABB for *P. purpureum.* Chen and Yang (1990) reported meiotic irregularities in *P. glaucum* x *P. purpureum* hybrid including bridges and laggards at anaphase I. Tetrads, triads, pentads and a special type of pentad composed of a tetrad and a small cell occurred. Normal pollen was rare. Small porate grains with a double wall were observed.

In a study by Cheng *et al.* (1989), the 2n pollen formation in hybrid napier grass were assessed for pollen fertility and seed set. The hybrid plant, which produced 2n pollen, had pollen fertilities ranging from 15% to 50%. The average number of seeds per panicle ranged from 1 to 16. Most hybrid progenies observed were amphidiploid (2n=42) hexaploids. Cytological and histological studies showed that 2n pollen formation occurred due to meiotic abnormalities as found by Chen and Yang (1990).

Triploid hybrids from crosses between diploid pearl millet and tetraploid napier grass produced high yields of high quality forage but were sterile. The chromosomes of these sterile F_1 hybrids were doubled using colchicine, but the female amphihexaploid were as not desirable forage types as the sterile triploids (Hanna *et al.* 1984).

To get a fertile hybrid, induced hexaploid was formed by giving colchicine treatment to the triploid hybrid (Gonzalez and Hanna 1984). This hexaploid hybrid contained 2n=42 with AAA'A'BB genomes, was male and female fertile and produced seeds.

Techio *et al.* (2005) studied chromosome pairing and the degree of genetic relationship between *P. purpureum* and pearl millet. The genomic relative affinities were calculated based on the evaluations of chromosome pairing of 50 cells in diakinesis, in which univalent, ring and rod (open) bivalent and trivalent figures were quantified. The pairing relationships estimate showed that pearl millet M24 and elephantgrass BAG 75 accessions, progenitors of F94-60-01, presented the major genetic proximity among their chromosome complement.

Hanna and Ruter (2005) registered *Princess* and *Prince* as new dwarf and semi dwarf, respectively, purple-foliaged Napier grass (*P. purpureum*) cultivars. They

were not recommended for frost-free subtropical areas but could be used as cold-hardy perennials in USDA as summer annuals further north.

P. purpureum has also been used as bridging species in a cross between *P. glaucum* and *P. squamulatum* for the development of apomictic pearl millet. Double cross hybrids (2n=42) produced by crossing a *P. glaucum* x *P. purpureum* amphiploid with a pearl millet x *P. squamulatum* interspecific hybrid which was then backcrossed with pearl millet (2n=28). In BC_4 generation obligate apomicts were obtained (Dujardin and Hanna 1989b).

Similarly, trispecific hybrids obtained by crossing strain 7418 of pearl millet x *P. purpureum* amphidiploid (2n=42) with *P. squamulatum* (2n=54) were studied to investigate the inheritance of apomixis. Morphological results and isozyme patterns were intermediate to the between the parents or more like *P. squamulatum*. Hybrids possessed 46-51 chromosomes, and showed meiotic abnormalities like delayed separation of bivalents, unequal segregation of multivalents, laggards and chromatid bridges at anaphase I. some hybrids, especially aneuploids, were matromorphic and had high seed set, stable chromosome number and uniform progeny with apomictic embryo sacs (Cheng and Cheng 1994).

Though there are several reports on interspecific hybridization between *P. glaucum* and related species, details on genome relationships is available only between *P. glaucum* and *P. purpureum*. The main reason for this might be that these two species are easily crossable and have morphological similarities as they belong to the same subsection Penicillaria and same gene pool.

2.4.4.3. Interspecific Hybridization with the species of tertiary gene pool

P. glaucum* x *P. schweinfurthii

Pennisetum schweinfurthii Pilger (2n = 2x = 14), a wild relative belonging to secondary gene pool of pearl millet (*P. glaucum*), has many desirable characters like rust resistance, bold grain size that could be transferred for pearl millet improvement utilizing interspecific hybridization. Till date, the hybrids between *P. glaucum* (2n=2x=14, 2n=4x=28) and diploid *P. schweinfurthii* have been reported to be highly sterile and difficult to maintain. Nagesh and Subrahmanyam (1996) obtained a hybrid between *P. glaucum* (2n=2x=14) x *P. schweinfurthii* (2n=2x=14) by embryo culture, was an annual, male sterile, partially female sterile and morphologically intermediate to both species. The hybrid developed with *P. schweinfurthii* as female

parent was resistant to rust as *P. schweinfurthii* was. The plants were tall and healthy, with thick stem and broad green leaves and with inflorescence of intermediate morphology between *P. schweinfurthii* and *P. glaucum.* At metaphase I, majority of the chromosomes remained univalents with occasional one or two rod bivalents. At anaphase I, unequal distribution of chromosomes was common. Selfing and crossing with fertility restorer line of *P. glaucum* was not successful.

Marchais and Tostain (1997) reported interspecific hybrids between *P. glaucum* and *P. schweinfurthii.* Both diploid and tetraploid pearl millet ovules showed the presence of hybrid zygotes after pollination with *P. schweinfurthii* at rates ranging from 25% to 45%. The diploid pearl millet x *P. schweinfurthii* hybrid zygotes often developed almost normal seed giving, without embryo rescue, totally sterile plants. The tetraploid pearl millet x *P. schweinfurthii* hybrid embryos were normal but the endosperm was severely defective. A hybrid obtained by embryo rescue was totally sterile. A diploid pearl millet x *P. schweinfurthii* amphidiploid was obtained by somatic embryogenesis associated with colchicine treatment during callogenesis. This amphiploid plant was male sterile, but gave many seeds when pollinated by a tetraploid pearl millet, few seeds when pollinated by a diploid millet and no seeds when pollinated by *P. schweinfurthii.* The same amphiploid plant gave about 4 seeds per panicle when pollinated by the F_1 pearl millet x *P. squamulatum* hybrid.

After post-zygotic abortion, another mechanism of reproductive isolation has been encountered with *P. schweinfurthii:* complete F_1 sterility due to the absence of homology between the chromosomes of *P. glaucum* and those of *P. schweinfurthii.* The RAPD fingerprinting of the F_1 hybrid plants exhibited 26 *P. schweinfurthii*-specific bands and 19 *P. glaucum*-specific bands. The resulting abnormal meiosis has been well described by Hanna and Dujardin (1986). Chromosomes paired mainly as bivalents at metaphase I for all accessions. Up to six bivalents were observed but because of the similarities of chromosome morphology of the two species, it could not be determined whether bivalents were due to intra or intergenomal pairing. The rod bivalents observed in these interspecific hybrids indicate some residual homology between the genomes of the two species. The amphiploid plant created with pearl millet and *P. schweinfurthii* has the structure (2n=28, genome GGSS). The backcrosses to diploid to tetraploid pearl millet will produce respectively GGS and GGGS plants probably male sterile and female fertile only with pollen from tetraploid millet. The creation of addition lines will be

hampered by the difficulty of a return to the diploid level. However, the backcross to the hybrid with *P. squamulatum* (genome GGSqSqSq) will perhaps be of some help in that respect.

Hanna and Dujardin (1986) reported interspecific hybrids between *P. glaucum* and *P. schweinfurthii*. *P. schweinfurthii* is the only *Pennisetum* species, other than pearl millet, reported to have 2n=14 chromosomes and an annual growth habit. Its chromosomes are similar in size but non homologous to those of pearl millet. The high male and female sterility, low bivalent formation at meiosis, and distinguishing morphological characteristics resulting in the two species being placed in different *Pennisetum* section indicate that these two species are not closely related. However, the occurrence of up to six bivalents in some microsporocytes, indicate possible residual homology between the genomes of the two species.

P. glaucum* x *P. squamulatum

P. squamulatum Fresen. (2n=54) is a wild species belonging to the tertiary gene pool of pearl millet. It contains many desirable characters like perenniality, apomixis, disease resistance, tolerance to drought and frost, etc. which could be transferred to pearl millet. Polyploid *Pennisetum* species often do not show strict bivalents pairing at meiosis (Dujardin and Hanna 1984). Although mostly bivalents were observed in *P. squamulatum*, univalents, trivalents, quadrivalents and hexavalents also were present (Akiyama *et al.* 2004). Roy *et al.* (2003) identified a new cytotype (2n=56) and reported regular disjunction of chromosomes at metaphase and anaphase of meiosis I. Occasional univalents and laggards were also seen. The plant shed abundant stainable pollen. A proposal for 2n=8x=56, with x=7 and has been registered (Roy *et al.* 2006).

Crosses between *P. glaucum* and *P. squamulatum* or *P. purpureum* have been relatively easy to make even though *P. squamulatum* has been reported to have a different basic chromosome number than the other 2 species (9 vs. 7) and to be hexaploid ($2n = 6x = 54$). Results obtained by Akiyama *et al.* (2006) had shown that chromosome number of *P. squamulatum* is $2n = 56$. By using centromeric and 18S-5.8S-26S rDNA probes as molecular cytological markers, the authors showed that *P. squamulatum* is most likely octoploid with a basic chromosome number of 7 ($2n = 8x = 56$) and may belong to the secondary gene pool of *Pennisetum*.

Patil *et al.* (1961) obtained a hybrid from a cross between *P. glaucum* (n=7) and *P. squamulatum* (n=27) which was partially fertile and had 2n=41

chromosome number apparently from unreduced female gamete of *P. glaucum*. At meiosis, they observed 16_{II} $+9_{I}$ in which seven bivalents were formed by the pairing between

P. glaucum and *P. squamulatum* chromosomes and the rest within the *P. squamulatum* complement.

Dujardin and Hanna (1983, 1989a) did not get any seed when diploid (2n=14) cytoplasmic male sterile line Tift 23AE was pollinated with *P. squamulatum* (2n=56). Partially fertile hybrids between tetraploid *P. glaucum* (2n=4x=28) and *P. squamulatum* were obtained and it was suggested that there is partial homology between the 'A' genome of pearl millet and one or more genomes of *P. squamulatum*. Studies on species relatedness of the *Pennisetum* gene pool using isozyme, storage protein, and DNA markers have confirmed cytogenetic observations of partial homology between *P. squamulatum* and the pearl millet genome (Chowdhury and Smith 1988, Lagudah and Hanna 1989, 1990).

New genome combinations were produced between tetraploid pearl millet (2n=4x=28) and *P. squamulatum* by pollinating a sexual interspecific F_1 hybrid (2n=41) with an apomictic 56-chromosome derivative from another F_1 interspecific hybrid (2n=41). Fifteen F_1 hybrids with 2n=47 or 48 resulted from these crosses. All were perennial, morphologically similar to or taller than their parents, showed irregular metaphase I chromosome behavior, and were partially male and female fertile. These plants reproduced sexually by facultative apomixis or by obligate apomixis. This cross allowed the increase of pearl millet genome in the new hybrids while introducing apomictic reproduction into several genotypes (Dujardin and Hanna 1990).

Partial male and female fertile F_1 were obtained between diploid pearl millet and *P. squamulatum* (2n=54). The BC generations of these hybrids were more fertile (Marchais and Tostain 1997). As the number of backcross generations increased, the probability of obtaining apomictic progeny decreased probably because aneuploid gametes are produced by BC plants with a high frequency of pearl millet chromosomes and an alien chromosome with the gene(s) controlling apomixis. Alien or extra chromosomes are usually transferred through the pollen at a reduced frequency. This indicates that genes controlling apomixis in *P. squamulatum* are restricted to a small portion of the genome, possibly to one single pair of chromosomes. This agrees with

other reports, which indicate that apomixis is controlled by one or few gene(s) (Hanna *et al.* 1973, Asker 1980, Bashaw 1980, Savidan 1982, Nogler 1986).

Crosses and backcrosses between tetraploid pearl millet (2n=4x=28) and hexaploid, apomictic *P. squamulatum* (2n=6x=54) have produced the most encouraging results in the interspecific transfer of the genes controlling apomixis from *P. squamulatum* to pearl millet. Dujardin and Hanna (1985a) produced BC_1 hybrids between pearl millet and *P. squamulatum.* Backcross to tetraploid pearl millet (2n=4x=28) resulted in BC_1 progenies with somatic chromosome numbers ranging from 2n=32 to 2n=39 with a mode of 2n=35 chromosomes. These hybrids were perennial and resembled pearl millet in morphological characteristics. Dujardin and Hanna (1989b) used a double cross hybrid involving pearl millet x *P. squamulatum* and pearl millet x *P. purpureum* to transfer apomixis derived from *P. squamulatum* into a 29-chromosome backcross derivative in an autotetraploid pearl millet background. This BC_3 hybrid was an annual vigorous bunchgrass which was 1.5 m tall and produced more tillers and panicles per plant when compared with pearl millet. Cytologically, most of the *P. squamulatum* genome was shown to be eliminated. However, similar morphology of some chromosomes of pearl millet, *P. purpureum* and *P. squamulatum* made it difficult to verify the genomic composition of the 29-chromosome apomictic backcross derivative. Hanna *et al.* (1993) produced BC_4 generation from the pearl millet x *P. squamulatum* cross. Six out of seven BC_4 plants produced some progeny that formed only aposporous embryo sacs. This study indicated that it is possible to produce apomictic pearl millet.

By successive backcrossing, the chromosome carrying gene(s) of apomixis has been transferred to the cultivated pearl millet, but the plant resulted with severely defective endosperm and reduced grain size (Dujardin and Hanna 1985a, 1989b, Hanna *et al.* 1993). Apomixis has been genetically mapped in *P. squamulatum* and shown to be linked with 12 molecular markers assayed as sequence-characterized amplified regions (SCARs; Ozias-Akins *et al.* 1998). As the 12 SCAR markers show no recombination with the trait, the linkage block has been termed the Apospory-Specific Genomic Region (ASGR) and the apospory is under the dominant control of an ASGR conserved in at least two grass species, *Pennisetum squamulatum* and *Cenchrus ciliaris* (Ozias-Akins *et al.* 1998, Roche *et al.* 1999).

Roche *et al.* (2001) presented evidence that in a reciprocal cross between sexual pearl millet and an apomictic F_1 (*P. glaucum* x *P. squamulatum*) the ASGR was not transmitted at the same rate.

By using FISH it has been determined that a single chromosome of *P. squamulatum* is sufficient for the transmission of apomixis and molecular markers linked to the trait in pearl millet (Goel *et al.* 2003). Further, fine mapping of the ASGR locus and its synteny with rice has been studied by Akiyama *et al.* (2004, 2005), Goel *et al.* (2006) and Gualtieri *et al.* (2006).

P. glaucum* x *P. setaceum

Apomictic inter-specific hybrids between cytoplasmic male sterile pearl millet (sexual, 2n=14) x *P. setaceum* (Forssk.) Chiov. (apomictic 2n=27) gave shriveled seeds and 3 perennial hybrids were produced by these seeds containing 2n=25 and 24 (Hanna 1979). The hybrid resembled the setaceum parent and in meiosis he could identify at least three bivalents based on the size difference in chromosomes due to pairing between the setaceum and the glaucum chromosomes.

The apomictic character of male parent was expressed in the hybrid. Chromosome elimination was found in somatic divisions, which would have an advantage for transferring germplasm from *P. setaceum* to *P. glaucum*. It was suggested that irradiation of F_1 plant can produce translocation between the genome and then recovering the pearl millet genome with pieces of the *P. setaceum* genome from tillers (Dujardin and Hanna 1989a). Dujardin and Hanna (1989a) observed that complete male sterility and obligate apomictic reproduction in these hybrids do not allow any further progress for transferring germplasm from *P. setaceum* to pearl millet.

P. glaucum* x *P. orientale

A sterile hybrid ($2n$=16) between *P. glaucum* (n=7) and *P. orientale* (n=9) was obtained by Patil and Singh (1964). Attempts have been made to hybridize *P. orientale* with pearl millet by various workers (Hanna and Dujardin 1982, Zadoo and Singh 1986). Jauhar (1973, 1981b) reported that part of *P. orientale* genome is partially homologous with part of glaucum genome.

An interspecific hybrid involving *P. glaucum* (2n=14) x *P. orientale* (2n=18) was backcrossed to *P. glaucum* using the hybrid (2n=16: 7'A' +9'O') as the female parent. Pollen mother cells of BC_1 plants contained a complement of 14 'A' +9'O' chromosomes. The BC_2 plants obtained by further backcrossing to *P. glaucum* had

21'A' +9'O' chromosomes revealing another addition of the *P. glaucum* genome. (Zadoo and Singh 1986)

In an interspecific hybrid involving *P. glaucum* and the tetraploid cytotype of *P. orientale* (2n=36), 7'A' and 18'O' chromosomes had been observed in F_1 hybrids by Hanna and Dujardin (1982). Out of 16 BC_1 plants raised in this cross, seven plants with 2n =23 (14'A'+ 9'O'), five plants with 2n=27 (7'A'+20'O'), and four plants with 2n=32 (14'A'+18'O'), were observed. Only the last category could be ascribed to unreduced gametes.

According to Zadoo and Singh (1986), the occurrence of a wide array of euploid chromosomal races in *P. orientale* other than the diploid 2n = 18, e.g. 2n = 27, 36, 45 and 54, suggests the operation of a genetic mechanism for the production of unreduced gametes, expressed in hybrid background. Such mechanism for unreduced female gamete formation in *Pennisetum* and its expression in interspecific hybrids might help in explaining the origin of higher polyploids in this genus. Hybrids were also obtained by Dujardin and Hanna (1989a), between diploid pearl millet (2n=14) and tetraploid *P. orientale* L. C. Rich. (2n=4x=36) and between tetraploid pearl millet and tetraploid *P. orientale.* The lack of recombination between the genomes of the two species was the limiting factor in germplasm transfer.

Nagesh and Subrahmanyam (1996) used embryo rescue technique to obtain viable progeny between *P. glaucum* (L.) R. Br. and *P. orientale* (2n=2x=18). Two *P. glaucum* spikes were pollinated with pollen from *P. orientale* which resulted in 40 seeds. Eighteen of the 36 embryos cultured produced F_1 hybrids which were vigorous and resembled the male parent. These hybrids had the expected 16 chromosomes (7 of glaucum and 9 of orientale). At metaphase I univalents were prevalent. These F_1 hybrids were sterile and could be propagated vegetatively.

P. glaucum* x *P. ramosum

Dujardin and Hanna (1989a) failed to get any interspecific hybrid between *P. glaucum* and *P. ramosum* (2n=2x=10), although germinating pollen grains were abundant on the stigmas of pearl millet. They suggested to overcome cross compatibility barriers between the species to get successful interspecific hybrids. In another study, Nagesh and Subrahmanyam (1996) used embryo rescue technique to obtain viable progeny between *P. glaucum* (L.) R. Br. with *P. ramosum* (2n=2x=10). Pollinations of pearl millet with *P. ramosum* did not give any viable progeny. Crosses

between pearl millet lines and *P. ramosum* (2n=2x=10) were observed for the frequency and development of zygotes, the possibility of embryo rescue, and the fertility of F_1 hybrids obtained (Marchais and Tostain 1997). 8% of the ovules from diploid millet x *P. ramosum* crosses showed small embryos which could not be rescued. However, 59% of the ovules from tetraploid pearl millet x *P. ramosum* crosses showed well-developed embryos that were easy to rescue 14 days after pollination. The F_1 hybrids obtained were male sterile but female fertile when pollinated by diploid pearl millet. These hybrids were morphologically similar to their male parent i.e. *P. ramosum.* The hybrid constitution of these plants was also ascertained by the 9 RAPD primers. These 9 primers showed 31 clear bands specific to *P. ramosum* and 29 clear bands specific to *P. glaucum.* They have suggested that post-zygotic abortion of pearl millet x *P. ramosum* hybrids is more severe with diploid than with tetraploid pearl millet.

These F_1 hybrids between tetraploid pearl millet (2n=28, genome GGGG) and *P. ramosum* (2n=10, genome RR) backcrossed to pearl millet produced plants with a probable genomic structure including the 7 pairs of G chromosomes and some individual R chromosomes. Considering the slight male fertility of F_1 hybrids, the recovery of some male fertile backcross plants (GGR or GG-) was expected with potential to produce addition lines of pearl millet with *P. ramosum* chromosomes.

2.4.4.4. Crosses of *P. glaucum* with other species of tertiary gene pool: Dujardin and Hanna (1989a) observed pollen from *P. pedicellatum* (2n=6x=54) and *P. polystachyon* (2n=6x=54) to germinate on the stigmas of diploid pearl millet but resulted in only shriveled miniature seeds that did not germinate. The partial seed development in diploid pearl millet x *P. pedicellatum* or *P. polystachyon* crosses indicate that embryo culture might be useful for recovering interspecific hybrids. Mutagene treatments with X-rays in *P. pedicellatum* (Saran and Narain 1982), as well as treatments with other crops and other mutagens, in order to break through the apomictic barrier, had no effect on the mode of reproduction, but changed at most the morphology. Detailed cytological analysis in tetraploid and hexaploid cytotypes of *P. pedicellatum* has been conducted (Zadoo 1986). A new cytotype of *P. pedicellatum* (*2n=8x=72*) was identified by Zadoo *et al.* (1997), and has been registered (Zadoo *et al.* 2006).

P. mezianum is the only *Pennisetum* species with basic chromosome number x=8. Dujardin and Hanna (1989a) failed to get any interspecific hybrid between *P. glaucum* and *P. mezianum* (2n=4x=32), although germinating pollen grains were

abundant on the stigmas of pearl millet. Nagesh and Subrahmanyam (1996) crossed *P. glaucum* (2n=2x=14) with *P. mezianum* (2n=4x=32) and used embryo rescue technique to obtain viable progeny. The hybrid plant thus obtained, resembled its corresponding pollen parent and was perennial and propagated vegetatively like male parent. Interestingly, F_1 plants showed difference in their pigmentation ranging from yellowish green to complete green and this was observed seasonally (during winter). Unlike their parents which flower all through the year, the F_1 hybrids flowered only during October to February. F_1 plants had the expected 23 chromosomes (16 from *P. mezianum* and 7 from *P. glaucum*). Very little or no pairing of chromosomes was observed during meiosis. The hybrids had low pollen fertility (17.5%).

Burson and Hussey (1996) reported that most $2n + n$ F_1 hybrids between *Pennisetum flaccidum* Griseb. and *P. mezianum* Leeke were more winter hardy and produced more forage than the $n + n$ F_1 hybrids. These findings demonstrate that the fertilization of an unreduced egg has potential for producing superior apomictic germplasm. However, the unpredictability and low frequency of this event limits its usefulness for improving apomictic species. Dujardin and Hanna (1989a) attempted cross between *P. glaucum* (2n=14) and *P. macrourum* (2n=36), but failed to get any interspecific hybrid although germinating pollen grains were abundant on the stigmas of pearl millet. Gildenhuys and Brix (1958, 1961a, b) produced a single hybrid with $2n$=21 chromosomes between diploid *P. glaucum* and *P. dubium* (n=14). They suggested no homologous relationships between the parental genome and the lack of any pairing was due to unfavorable gene combination in the hybrid.

P. schweinfurthii* x *P. purpureum

Vidhya and Khan (2003) reported a hybrid between *P. schweinfurthii* (2n=14) and *P. purpureum* with 2n=28 chromosomes. In the light of the earlier reports from the cross *P. glaucum* x *P. schweinfurthii*, it was suggested that an unreduced gamete of *P. schweinfurthii* might have combined with a normal gamete of *P. purpureum* to give rise to the 2n=28 hybrid. It was also suggested that the genomic constitution of the hybrid might be A'BBB and the 14 bivalents observed might be due to pairing of 7 chromosomes of the B genomic compliment and the other 7 bivalents may be due to intergenomic residual homology. With the limited information, the authors had assumed that *P. glaucum* (AA) and *P. schweinfurthii* (BB) might be the putative parents of *P. purpureum* (AABB).

P. glaucum* x *Cenchrus ciliaris

Mohindra and Minocha (1991) studied the pollen pistil interactions and interspecific incompatibility in *Pennisetum.* They observed rapid growth of the pollen tubes in *P. glaucum* x *P. cenchroides* (or *Cenchrus ciliaris*). But many pollen tubes stopped in the hairy region of the style due to swelling of their tips and was responsible for low crossability in this cross. No seed set was observed in this cross. According to Heslop-Harrison (1982), it appeared that an ovarian as well as stylar incompatibility system exists in the *P. glaucum* x *P. cenchroides* crosses.

The diploid pearl millet x *Cenchrus ciliaris* crosses gave high frequencies of proembryos with large undifferentiated endosperm very similar to the situation observed in the diploid pearl millet x *P. squamulatum* crosses. Considering the post-zygotic abortion in diploid pearl millet x *Cenchrus ciliaris* hybrids and in diploid pearl millet x *P. squamulatum* hybrids, it may be hoped that a tetraploid line could give plenty of partially fertile hybrids with *C. ciliaris* (Marchais and Tostain 1997).

A genome map of buffelgrass (*Pennisetum ciliare* (L.) Link syn. *Cenchrus ciliaris* (L.) was studied by Jessup *et al.* (2003). Maternal and paternal maps were constructed with restriction fragment length polymorphism (RFLPs) segregating in 87 F_1 progeny from an interspecific cross between two heterozygous genotypes. In the results, approximately 70 to 80% of the buffelgrass genome was covered, and the average marker spacing was 10.8 and 11.3 cM on the respective maps. Preferential pairing was indicated between many linkage groups, which supports cytological reports that buffelgrass is a segmental allotetraploid. Comparision of interval lengths in 15 allelic bridges indicated significantly less meiotic recombination in parental gametes.

3. MATERIALS AND METHODS

3.1. MATERIALS:

The present investigation was carried out at Indian Grassland and Fodder Research Institute Jhansi, India during 2003- 2007. The present study was carried out on various *Pennisetum* species and interspecific hybridization was attempted to produce new hybrids. The details of procurement of material, methodology for morphological data recording, cytological investigations, biochemical analysis, induction of colchiploidy, intraspecific and interspecific hybridization, advancement of generations and analysis of data is as follows:

3.1.1. Procurement of material: The wild *Pennisetum* species belonging to primary, secondary or tertiary gene pool were procured from various sources viz. Indian Grassland and Fodder Research Institute, ICRISAT and USDA (Table 3.1). These were established and maintained in the field following appropriate agronomic practices. Diploid and tetraploid pearl millet lines available at IGFRI were also utilized. Species and pearl millet lines were maintained by self-pollination while the male sterile lines were maintained by their respective maintainers.

The F_1 hybrids between pearl millet and *P. squamulatum*, and F_1 and BC_1 plants between pearl millet and *P. orientale* were obtained from IGFRI, Jhansi.

3.1.2. Wild species characterization: The cultivated and wild *Pennisetum* species procured from various sources were characterized for their morphological, cytological and biochemical attributes. The details of various species used in the present study are given in Table 3.1.

3.1.3. Induction of polyploidy: Attempts were made to induce polyploidy in two primary gene pool species viz. pearl millet (diploid male sterile lines and tetraploid lines) and *P. violaceum*, and two tertiary gene pool species viz. *P. schweinfurthii* and *P. ramosum*. All having x=7 basic chromosome number except *P. ramosum* that had x=5 basic chromosome number (Table 3.2). Colchicine was used for the induction of polyploidy.

3.1.4. Interspecific hybridization: The wild species procured were used in the interspecific hybridization. Figure 1 provides synopsis of interspecific hybridization carried out in the study.

Intragenomic: Crosses were attempted between the species belonging to the same genome and ploidy i.e. intragenomic and intraploidy and at same genome at different ploidy i.e. intragenomic and interploidy.

Intragenomic and intraploidy: In this category crosses of species having same ploidy i.e. pearl millet (2n=2x=14) with various accessions of *P. violaceum* (2n=2x=14) and *P. mollisimum* (2n=2x=14) were included.

Intergenomic: *P. glaucum* crossed with *Pennisetum* species with different genomes as well as different ploidal status.

Intraploidy: Crosses were attempted between species of same ploidy in which diploid pearl millet (both male fertile and male sterile) was crossed with various accessions of diploid *P. schweinfurthii* (2n=2x=14).

Interploidy: Hybridization was carried out between pearl millet with different wild species at different ploidy status. Interploidy crosses can be further divided into three categories. First category includes crosses between diploid pearl millet with species of tertiary gene pool, while the crosses of tetraploid pearl millet with the species of tertiary gene pool were included in second category. Third category includes the crosses between the wild species (other than pearl millet).

3.1.5. Selection of parents and compatibility studies: Suitable accessions belonging to species with x=5, 7 and 9, such as *P. ramosum, P. schweinfurthii, P. squamulatum, P. orientale, P. pedicellatum, P. polystachyon, P. flassidum, P. orientale, P. hoheneckeri,* were selected for attempting crosses. Diploid male sterile lines and tetraploid lines of pearl millet were used for attempting crosses with diploid/tetraploid species. For polyploid species, tetraploid pearl millet lines were used. Diploid species were also crossed with tetraploid pearl millet and vice versa (Table 3.3).

3.1.6. Advancement of generations:

Pearl millet x *P. violaceum*: F_1, F_2 and BC_1 plants were developed. The hybrids were characterized morphologically and cytologically.

Pearl millet x *P. squamulatum*: F_1 hybrids between two accessions of tetraploid pearl millet (IG 99-748 (= T1) and IG 2000-01 (= T2)) and three accessions of *P. squamulatum* (IG 98-360 (= MA), IG 98-361 (= MB) and IG 2000-36 (= MC)) developed at IGFRI Jhansi (Kaushal *et al.* 2003a) were used in crossing for advancement of generations. Two F_1 hybrids (H1 sexual and H2 apomictic) were crossed with tetraploid pearl millet (T1 and T2) in all possible directions i.e. H1 x T1, T1 x H1, H1 x

T2, T2 x H1, T1 x H2 and T2 x H2 to produce BC_1 generations. F_1 and BC_1 generations were grown and characterized for morphological, cytological and isozymic attributes. Few F_2, and sib plants were also cytologically analyzed.

Pearl millet x *P. orientale*: F_1 and BC_1 of this interspecific cross (Zadoo and Singh 1986), being maintained at IGFRI were cytologically analyzed and new interspecific crosses were attempted to these already existing hybrids. The new hybrids produced were analyzed cytologically and morphologically.

3.2. METHODS:

The different methodologies used in the present study were as follows:

3.2.1. Establishment of the material: The pearl millet lines, different accessions of wild *Pennisetum* species, and the interspecific hybrids were sown in field/pots and were established.

Pearl millet lines (male sterile and maintainers; diploid and tetraploids), wild *Pennisetum* species, as well as their hybrids were characterized for morphological, cytological and biochemical attributes as detailed under section 3.2.2.

On the basis of cytological, biochemical and morphological attributes, appropriate species and accessions were identified and utilized in crossing program. Artificial pollination was attempted between selected species and pearl millet lines. Diploid accessions were crossed with male sterile pearl millet lines whereas wild accessions with higher ploidy levels were crossed with tetraploid lines. Staggered sowing of pearl millet lines was done for synchronization of flowering between pearl millet and the wild species. In case of crosses exhibiting post-fertilization incompatibility barriers, embryo rescue was also utilized.

3.2.2. Characterization of the material:

3.2.2.1. Morphological observations: Morphological characters were recorded for the following characters at 50% flowering stage. These characters were grouped as qualitative and metric traits. The observations were recorded on three plants selected at random within a line.

Qualitative traits: The qualitative traits were recorded on the basis of visual observations.

1. **Habit:** The plants were characterized as annual (A), biennial (B) or perennial (P).

2. **Hairiness (presence or absence of hairs):**
    - **Node:** The hairiness of node was classified as hairy (H) or non-hairy (NH).
    - **Leaf base:** It was classified as hairy (H) or non-hairy (NH).
    - **Leaf sheath:** Either hairy (H) or non-hairy (NH).
3. **Node colour:** Node colour was characterized as yellow (Y), light violet (LV), violet (V) and Dark Violet (DV).
4. **Leaf base colour:** It was observed as yellow (Y), green (G), light violet (LV), violet (V) and dark violet (DV). In some plants, some leaves were with yellow base and some were with violet colour. So this was recorded as yellow + violet (Y+V).
5. **Leaf orientation:** Leaf orientation was recorded as erect (E), semi-drooping (SD) and drooping (D).
6. **Leaf colour:** It was recorded as light green (LG), green (G) and dark green (DG).
7. **Awn colour:** Awns colour was classified as white (W), cream (C), yellow (Y), green (G), light violet (LV), violet (V) and dark violet (DV).
8. **Stigma:**
    - **Colour:** It was classified as white (W), light violet (LV), violet (V) and dark violet (DV).
    - The stigma was either bifid (B) or trifid (T).

Metric traits:

1. **Plant height (cm):** The plant height was measured from the base to the top of the main tiller.
2. **Number of tillers:** Total number of tillers present in the plant was noted.
3. **Stem diameter (cm):** Stem diameter was measured from the middle portion of $3^{rd}/4^{th}$ node with the help of vernier calliper.
4. **Number of leaves/tiller:** The total number of leaves on the main tiller was noted.
5. **Number of nodes/tiller:** The total number of nodes on the main tiller was noted.
6. **Internode length (cm):** The length of the 3^{rd} internode from the base of main tiller was measured.
7. **Leaf characterization:**
    - **Flag leaf length (cm):** It was measured from the attachment of the leaf to the sheath to the tip of the leaf.

- **Flag leaf width (cm):** The width of the same leaf was measured at the point of maximum width.
- **3rd leaf length (cm):** The 3rd leaf from the top of the tiller was measured.
- **3rd leaf width (cm):** The width of the same leaf was measured at the point of maximum width.

8. **Penduncle length (cm):** Peduncle length was measured from the last node of stem to the end of the inflorescence.
9. **Spike:**
   - **Length (cm):** From the starting point of spikelet emergence to the apex of panicle was measured.
   - **Width (cm):** The middle part of the spike was measured.
   - **Number of florets/spikelet:** The total number of florets (either hermaphrodite or unisexual) was recorded, per spikelet.

3.2.2.2. Cytological observations: Cytological studies were carried out on the various accessions of *Pennisetum* species (wild and cultivated) belonging to primary, secondary or tertiary gene pools, on the interspecific hybrids produced, on the advanced generations of already existing interspecific hybrids and on the induced colchiploids of *Pennisetum* species produced in the present programme. For meiotic studies, the young developing panicles at boot stage were collected and fixed in Carnoy's fluid (3 ethanol: 1 glacial acetic acid). Fixation was done between 9:30 am to 10:30 am. The panicles were fixed for at least 24 hours. The anthers were dissected out and were squashed in a drop of 2% acetocarmine solution on microscopic slides. Meiosis was studied in this temporary smear, for stages of meiosis I and meiosis II. The different chromosomal associations at diakinesis and/or metaphase I in the species as well as the hybrids and the BC progeny was recorded. Most of the species were polyploids, so various multivalent associations were observed at diakinesis. The chiasmata frequency and range were also recorded. The chromosomal segregation and abnormalities like laggards, chromosomal bridges, micronuclei, in anaphase I and later stages were observed using Olympus CX40 or Leica DM2000 microscope. Photomicrography of informative cells was performed with Olympus SC35 or Canon PowerShot S70 camera.

Pollen fertility was estimated on the basis of stainability test. Freshly dehisced pollen grains were observed by staining with glycerocarmine (1 glycerin: 1

acetocarmine (2%)). Darkly stained and regular sized pollen grains were recorded as fertile and those partially stained, unstained, shrunken, or irregular shaped pollen grains were counted as sterile.

Preparation of reagents:

2% acetocarmine: Two grams of Carmine powder (LOBA Chemie) was added slowly to 100 ml of boiling 45% acetic acid and kept at shimmering condition for 30 minutes. This solution was then cooled, filtered using Whatman No. 1 filter paper and stored in airtight glass bottle.

Fixative: The material was fixed in Carnoy's fluid (3 parts absolute ethanol and one part glacial acetic acid). Traces of $FeCl_3$ were also added to this fixative for better staining.

3.2.2.3. Biochemical studies: Different accessions of *Pennisetum* species and the hybrids were compared for the four enzymes- Super oxide Desmutase (SOD, E.C. 1.15.1.1.), Esterase (EST, E.C. 3.1.1.2.), Peroxidase (POD, E.C. 1.11.1.7.) and Glutamate Dehydrogenase (GDH, E.C. 1.4.1.2.). EST, SOD and GDH were subjected to polyacrylamide gel electrophoresis (PAGE) in 'Genei' vertical migration chamber and POD was subjected to horizontal starch gel electrophoresis using discontinuous buffer system. The plate was connected with the buffer chamber. A constant current of 20 mA was given till the tracking dye crossed the stacking gel. Thereafter the current was increased to 40 mA till the tracking dye reached the bottom of the gel.

To estimate the genetic diversity, the bands were scored and numbered on the basis of their relative mobility towards anodal/cathodal ends.

Starch gel electrophoresis:

Preparation of stock solution and buffers:

1. Extraction buffer (0.1M, pH 7.5): 1.211gm of Tris (MW 121.14), 1 gm of polyvinyl Pyrrolidone (PVP MW 40,000) and 0.037gm of Ethylene diamine tetra acetic acid (EDTA MW 292.25) were dissolved in distilled water and pH was adjusted to 7.5 by adding drops of 1N HCl and final volume was made up to 100ml using double distilled water .

2. Bridge buffer (pH 8.65) Sodium borate buffer: 18.550gm of boric acid (MW 61.83) and 4.0gm of NaOH (MW 40.00) were dissolved in distilled water and pH was adjusted to 8.65 by adding drops of 1N HCl and final volume was made up to 1 liter using double distilled water.

Enzyme extraction: The leaf samples at the same stage of growth from different accessions were collected carefully. The crude extract from the leaves was prepared by homogenizing 1g of sample with 1ml of chilled Tris buffer (pH 7.5), in a pre-chilled pestle and mortar. The crude extract was filtered through muslin cloth and the filtrate was stored in the deep fridge (-20°C) in different vials. Each vial was thawed only once just before use.

Gel preparation: For gel preparation, 14% starch was found suitable for adequate polymerization. In a conical flask, 22.4 g of hydrolyzed potato starch was taken and 160 ml Tris buffer was added to it. The content was heated and was vigorously shaken while heating, till it became less viscous and translucent. The cooked starch was immediately poured on to a glass plate (12 x 12 cm) having 4 tiers of glass strips pasted on it. The tray was then covered with a glass plate in such a way that no air bubbles were trapped. The gel plate was kept to set at room temperature for 5-6 hrs.

Sample loading on the gel: The cover from the gel plate was removed with the help of a blade. The samples to be analyzed were soaked onto filter paper wicks (Whatman no. 3) of size 5mm x 8mm and inserted into the slots. Slots were made in the middle of the gel for peroxidase with the help of the stick cutter.

Electrophoresis: The gel plate was then placed in 'Genei' horizontal migration chamber. Bridge buffer was poured into the buffer chambers so that electrodes were completely dipped. The plate was connected with the buffer chamber with the help of filter paper bridge (Whatman no. 1.), which was earlier saturated with buffer. During the process, the migration chamber was placed in a refrigerator to avoid over heating of the gel. Electrophoresis was carried out at constant current of 20 mA for first 30 minutes followed with 40 mA till the front reached the other end. The gel plate was taken out and the glass stripes were removed from the side of the glass plate. Each gel was sliced thrice, horizontally, with the help of a copper wire, to obtain four slices of gel. The first slice was discarded and lower ones were used for staining.

Polyacrylamide gel electrophoresis (PAGE): PAGE analysis was done using Polyacrylamide vertical gel electrophoresis system.

Prepration of stock solutions and buffers:

1. Grinding buffer (pH=8.8): 0.6052 gm of Tris, 200 µl of mercaptoethanol, 0.168 gm of Ethylene diamine tetra acetic acid (EDTA) and 5 gm of sucrose were dissolved in

distilled water, pH was adjusted to 8.8 by adding drops of 1N HCl and final volume was made up to 100ml using double distilled water.

2. Acrylamide stock solution: 29.2 g of Acrylamide (MW 71.08) and 0.8g of N.N. methylene Bis Acrylamide (MW 154.17) were dissolved in double distilled water and final volume was made upto 100 ml. The solution was stored in an amber coloured bottle at 4°C.

3. Tris HCl buffer for separating (Resolving gel) (pH 8.9): 18.15 g of Tris dissolved in double distilled water and pH was adjusted to 8.9 by adding drops of 1N HCl. Final volume was made upto 100 ml using double distilled water.

4. Tris HCl buffer for stacking gel (pH 6.7): 6.1 g of Tris dissolved in double distilled water and pH was adjusted to 6.7 by adding drops of 1 N HCl. Final volume was made upto 100 ml using double distilled water.

5. Ammonium per sulphate (APS): 0.1 g of Ammonium per sulphate (MW 228.2) was dissolved in 100 ml of double distilled water.

6. Riboflavin solution: 40 mg of Riboflavin (MW 376.37) was dissolved in 10 ml of double distilled water and was filtered before use.

7. Running gel electrode buffer (pH 8.3): 2.8 gm of glycine and 600 mg of Tris HCl dissolved in distilled water and pH was adjusted to 8.3 by adding drops of 1 N HCl and final volume was made up to 1 liter using double distilled water.

8. Tracking dye: It was prepared by mixing 0.25% Bromophenol blue with 40% sucrose solution.

Enzyme extraction: 1 g of disease free and young leaves from required samples (at same stage of growth) were grinded in Tris buffer (pH 8.9) in 1:3 ratio (500 mg sample + 1500 µl buffer) in pre chilled pestle and mortar. The homogenate was collected in 1.5 ml microcentrifuge tubes and centrifuged at 12000 rpm for 20-25 minutes. The supernatant was collected in separate 1.5 ml microcentrifuge tubes and stored at –20° C.

Preparation of resolving gel: 40 ml resolving gel (10%) was prepared by adding Acrylamide (30%, 16 ml), Tris HCl (10 ml), H_2O (14 ml), Tetramethyl ethylenediamine TEMED (20 µl) and Ammonium per sulphate (10%, 200 µl). The gel solution was immediately poured in vertical gel casting unit and left for one hour for setting in undisturbed condition. At the top of resolving gel 5 ml of water was poured so that the gel does not get dried.

Preparation of stacking gel: 10 ml of stacking gel (5%) was prepared by adding Acrylamide (30%, 1.7 ml), Tris HCl (1.3 ml), H_2O (6.9 ml), Tetramethyl ethylenediamine (10 µl) and Ammonium per sulphate (50 µl). This gel solution was poured over the resolving gel after removing the top level of water. Pouring of stacking gel was immediately followed with placing the comb. This gel was left overnight. 50 µl of each sample was mixed with 10 µl of tracking dye and was loaded in different wells after removing the comb.

Electrophoresis: The gel plate thus prepared was placed in 'Genei' vertical migration chamber. Running gel electrode buffer was poured into the migration chambers so that electrodes were completely dipped. The plate was connected with the buffer chamber.

A constant current of 20 mA was given till the tracking dye crossed the stacking gel portion. After that the current was increased to 40 mA till the tracking dye reached the bottom of the gel.

Staining of the gel: For peroxidase the gel was stained by following method given by Veech (1969) and for SOD, Esterase and GDH, the methods as suggested by Wendel and Weeden (1989) were followed.

Prepration of staining solutions: Staining solutions were same for both starch gel electrophoresis and for polyacrylamide gel electrophoresis.

1. **Gel buffer- Tris citrate buffer (pH 8.65):** 9.206 g of Tris and 1.051 g of citric acid were dissolved in distilled water and final volume was made up to 1 liter.
2. **Acetate buffer (0.2 M, pH 5.6):** 27.216 g of Sodium acetate trihydrate and 2.6 ml of Acetic acid dissolved in distilled water and final volume was made up to 1 liter.
3. **Phosphate buffer (0.1 M, pH 6):**

   Monobasic: 31.2 g of sodium dihydrogen ortho-phosphate dissolved in 1 liter of distilled water.

   Dibasic: 28.4 g of Disodium hydrogen orthophosphate dissolved in 1 liter of distilled water.
4. **Stain solution for native protein:** 100 mg of Coomaise brilliant blue R-250 in 100 ml solution of 40 mg methanol, 10 ml acetic acid glacial and 50 ml distilled water.
5. **Destain solution:** 10 ml acetic acid glacial, 40 ml methyl alcohol and 50 ml distilled water, added to make 100 ml destaining solution.

Staining of the gel:

- **Peroxidase:** 100 mg of benzidine was dissolved by heating in 100 ml of 0.2 M acetate buffer (pH 5.6). After cooling the benzidine solution, 2 ml of 3% hydrogen peroxide was added at the time of incubation of the gel. After 10 minutes of incubation, blue bands appeared which turned brown later. The sites of peroxidase isozyme were stained by the oxygen released in the reaction, which oxidizes benzidine, a colourless compound to a coloured one.
- **Superoxide Desmutase:** The gel was stained in 100 ml of Tris HCl buffer (pH 8.65), in which Riboflavin (4 mg), Ethylene diamine tetra acetic acid (EDTA 2 mg) and Nitro blue tetrazolium chloride (NBT 20 mg) were added. The gel was incubated in dark for 30 minutes and then exposed to intense light for 1.5 hrs until bands appeared.
- **Esterase:** 68.5 ml of monobasic phosphate buffer, 31.5 ml of dibasic phosphate buffer and 100 ml distilled water was mixed to make phosphate buffer (pH 6.5). The gel was incubated in this 100 ml of 0.1 M phosphate buffer (pH 6.5) containing 32.5 mg of 1-napthyl acetate in 1 ml acetone and 50 mg of fast blue RR salt at room temperature for 60 minutes. The site of Esterase enzyme activity appeared as reddish brown to blackish bands on the gel.
- **Glutamate Dehydrogenase (GDH):** The gel was stained in 100 ml of Tris HCl buffer (pH 8), in which α glutamate, sodium salt (400 mg), $CaCl_2$ (100 mg) and Nitro blue tetrazolium chloride (NBT 20 mg), Phenazonium methosulphate (PMS 4 mg) and β-Nicotinamide adenine dinucleotide (NAD 20 mg) were added. The gel was incubated in dark for 30 minutes and then exposed to intense light for 1.5 hrs until dark blue bands appeared.
- **Native Protein:** Gel was washed and dipped in 10% blue brilliant (Coomaise brilliant blue) R-250 for 6-7 hours. Then placed in destaining solution again for 4-5 hours, until bands were clearly visible.

Zymogram preparation: The bands for different isozymes and proteins were drawn on graph paper at 1:1 ratio. The point of origin and front was also marked in order to calculate relative mobility of the bands. Selected gel plates were photographed.

Nomenclature of the bands: The bands were scored from the place of origin where samples were loaded to the end of front movements. The slowest band was considered as band 1 and subsequently bands were numbered based on the pooled zymogram for the

genus. In case of peroxidase, bands were named separately as anodal and cathodal bands and in both cases the movement of bands was considered from the place of origin where the samples were loaded.

3.2.3. Induction of polyploidy:

The diploid species *P. violaceum, P. ramosum, P. schweinfurthii* and male sterile lines of *P. glaucum* were given colchicine treatments for induction of tetraploidy and tetraploid pearl millet was given colchicine treatment for induction of octoploidy. Colchicine was used at different concentrations of 0.05%, 0.1% and 0.2% and durations 6 hrs, 12 hrs, 18 hrs and 24 hrs.

Seeds of the species for colchicine treatments were obtained from the germplasm collection maintained by IGFRI Jhansi (Table 3.2). Four types of colchicine treatments were given for induction of tetraploidy.

1. Seed treatment: The seeds of different accessions of these species were allowed to germinate on filter paper soaked with colchicine solution different concentrations (0.05%, 0.1% and 0.2%) and time (6 h and 24h) in petri plates (15 seeds per petri plate).

2. Shoot treatment: The seeds were allowed to germinate on filter paper soaked with water, in petri plates. After 4-5 days, when the seedlings were about 1 cm (shoot), the shoot was cut at the tip and wrapped in tissue paper soaked in colchicine solution at different concentrations (0.1% and 0.2%) and kept on a slide in such a way that only shoot remain in touch with colchicine and the root was left in water in the petri plate.

3. Seedling capillary treatment: Seeds were sown in pots in the month of August. When the seedlings were 14 days old, the tiller was cut off an inch below the growing point and capillary tubes filled with 0.05%, 0.1 % and 0.2% colchicine solution were placed over the cut end so as to fit tightly. The treatment was given for 24 hrs. The capillaries were removed and the seedlings were allowed to grow in pots.

4. Colchicine injection: The flowering tillers were treated with colchicine by injection treatment after 48 and 72 hours of pollination. The colchicine solution of different concentration (0.05%, 0.1 % and 0.2%) in addition to 2-4D was injected in the first internode (just below the peduncle).

To confirm induction of tetraploidy, initial screening of the treated plants was done by flow cytrometry of leaf samples. Then stomata of the suspected plants were observed and on flowering, plants were cytologically analyzed.

Flow cytometry: The genome size of colchicine treated plants and the BC_1 generation plants were determined by flow cytometry. Approximately 3 cm^2 young disease free leaf was chopped with a sharp razor blade, stained with DNA-specific fluoreschrome DAPI (100mM Tris-HCl, 5mM $MgCl_2$, 85mM NaCl, 0.1% Triton X 100 and 1µg/ml DAPI) and filtered through a 40 µm sieve. Suspended nuclei were detected with a Partec ploidy analyzer (PAI) flow-cytometer using the recommended setup for DAPI fluorescence. Each sample counted contained 2,000-4,000 fluorescent particles (Kaushal *et al.* 2007).

3.2.4. Interspecific hybridization: The pearl millet lines (male sterile, maintainer and tetraploid) and the wild *Pennisetum* species belonging to different gene pools and different ploidy status were used in intraspecific and interspecific hybridization. Crosses were made in both the directions i.e. using pearl millet as female as well as male parent (Table 3.3). Crosses were also attempted between the wild species given in Figure 1. The introgression of various alien characters like perenniality, high tillering, disease resistance, etc. from wild species to the cultivated pearl millet were observed in the BC_1 generations.

Harvesting and threshing: After one month of crossing the crossed spikes were harvested and kept for another 15 days. When the harvested spikes dried properly, they were threshed and the seeds were collected.

Data recording: The threshed seeds were counted and the data was recorded for self pollinated, cross pollinated or open pollinated seeds.

3.2.4.1. Hybridization: Developing spikes from the pearl millet lines female parents were covered with glycine/butter paper bags at appropriate stage prior to gynoecia appearance. Marking on selfing bags was done with pencil. Pollen from the desired male parents was collected in paper bags covered on previous day of pollination. Artificial crossing was done manually by dusting the pollen onto the spikes with most receptive stigma stage and covered again by the pollination bags. The pollination was carried out between 10 am to 12 pm in full sunshine and with appropriate amount of pollen in the month from October to January.

3.2.4.2. Embryo rescue:

Pollination: Pollination was done by conventional method as described in the previous section.

Media preparation: The inorganic salts (Murashige and Skoog 1962) were mixed in double distilled water supplemented with 3% sucrose and agar (0.7%). The composition of different media used in the study are given in Table 3.4 and Table 3.5.

Preparation of basal media: It was prepared by mixing appropriate combination of inorganic salts as mentioned in the Table 3.4 and 3.5. The salts were dissolved by adding one salt at a time. Precipitation was avoided by dissolving the inorganic Nitrogen source first. Growth hormones were added in different combinations for specific objectives before the addition of agar. pH of the media was adjusted to 5.8 using 1N NaOH. After the addition of agar and sucrose the medium poured into the test tubes and plugged with non-absorbent cotton wrapped in muslin cloth. The medium was autoclaved for 25 minutes at 15Lbs/sq. inch pressure and at 120°C temperature. Autoclaved medium was allowed to cool and kept in dark. After the solidification at room temperature the medium was used for culturing of the embryos.

Embryo culture media: It was used for the germination of the embryos. The inorganic salts (Murashige and Skoog 1962) were mixed along with appropriate growth regulators (Kinetin 0.5 mg/l and Naphthalene acetic acid 1 mg/l) and other additives like Casein hydrolysate (500 mg/l) in double distilled water.

LsP3 media: After few weeks the plantlets were transferred to LsP_3 media. In these inorganic salts (Philips and Collins 1984) were mixed in double distilled water along with growth regulators [L_2 + Naphthlene acetic acid (NAA 0.008 mg/l) and 6 Benzyl aminopurine (BAP 0.15 mg/l)].

Embryo culture: After pollination the crossed spikes were observed daily and after 15 to 18 days of pollination (just one day before the seed starts shriveling), the developing seeds were harvested and surface sterilization was done by immersing seeds in 0.1% mercuric chloride ($HgCl_2$) for 2-3 minutes. This was followed by 4-6 washings in sterile distilled water. Embryos were then excised under aseptic conditions under laminar flow and put in ½ MS media and embryo culture media. The cultured embryos were placed in dark at 25±2°C till germination.

After germination, the seedlings were provided with a day light cycle of 8/10 hours.

Hardening and field transfer: The tubes were taken out of culture room and kept at room temperature for 2-3 days. The plantlets were taken out of tubes washed carefully to remove the agar media. Extra precautions were taken at this stage so as to cause minimum damage to root or shoots.

3.2.5. Analysis of the data: The data gathered during the course of study was analyzed for genetic similarity between and within different species studied, compatibility behavior between different species, cytogenetical status of species as well as their hybrids with pearl millet and effect of alien introgressions in first and second generation hybrids.

Cluster analysis based on morphological characters:

The metric trait data recorded for various morphological traits in species and the hybrids were analyzed using non-Hierarchical Elucidian cluster analysis for grouping of genotypes (Spark 1973). All computation was done using the computer software SPAR 1 Release 1.1 (IASRI, New Delhi). The replicated data were averaged over replicates and then cluster analysis was carried out.

ANOVA and Chi square (χ^2) test using cytological data:

The Analysis Of Variance was calculated in the BC_1 progeny of pearl millet and *P. squamulatum* to observe some reciprocal cross differences if present in the BC_1 produced in all possible directions.

The χ^2 test of goodness of fit is of wide applicability to numerous problems of significance in frequency data. This test was primarily used for testing the agreement of observed frequencies with those expected upon a given hypothesis as, for instance, in comparing an observed frequency distribution with a theoretical one like the normal. For carrying out this test, we calculate, from the data, the quantity

$$\chi^2 = \sum \frac{(O-E)^2}{E}$$

where O stands for the observed and the E stands for the expected frequency in any particular class of the distribution and $\sum$ for the summation over all classes. It can be seen that for a complete agreement with the hypothetical distribution of the value of the x^2 of the score will be zero, but chance deviations are bound to occur and positive values of the score in sampling will be obtained due to sampling fluctuations. The test was applied in the cytological data of the BC_1 progeny of pearl millet and *P. squamulatum*.

Similarity matrix analysis and cluster based on isozyme bands:

A binary data matrix reflecting the presence or absence of specific isozyme band was generated for all accessions of *Pennisetum* species and the F_1 and BC_1 generation of pearl millet and *P. squamulatum*. Only unambiguously scored bands were used in the matrix.

The genetic similarities (GS) between line I and j were estimated using the formula of Dice (1945)

Gsij=2Nij (Ni+Nj)

where, Nij is the number of common bands between i and j and Ni is the number of band in I and Nj is the number of band in j respectively. A dendogram was prepared using the unweighted-pair-group method average (UPGMA) clustering procedure. All computation was done using the computer software NTSYS-PC version 1.60.

4. RESULTS

4.1. *PENNISETUM* SPECIES: The results of morphological cytological and biochemical studies are described below:

4.1.1. Morphological characterization:

Thirty-two accessions representing fifteen species were analyzed for their morphological characteristics. The data was recorded for both metric and qualitative traits.

4.1.1.1. Variability for quantitative traits (Table 4.1, Plate 1):

The various metric traits were subjected to statistical analysis to ascertain the level of genetic variability. The analysis of variance was calculated for the characters like number of tillers, height of main tiller, number of leaves, flag leaf length, flag leaf width, 3rd leaf length, 3rd leaf width, stem girth, number of nodes, internode length, peduncle length, spike length, spike width and number of florets per spikelet (Table 4.1, Plate 1).

The mean, coefficient of variation, genotypic coefficient of variation, phenotypic coefficient of variation, genetic advance values and heritability were observed for various traits. The results showed a considerable extent of variation for all the traits studied.

Cluster analysis: Non- Hierarchical Euclidian cluster analysis technique was used to determine the extent of variation/similarity among thirty two accessions based on fourteen morphological traits *viz.,* number of tillers, height of main tiller, number of leaves, flag leaf length, flag leaf width, 3rd leaf length, 3rd leaf width, stem girth, number of nodes, internode length, peduncle length, spike length, spike width and number of florets per spikelet. The analysis resulted in identification of six clusters (Table 4.2). Cluster 2 was the largest one with total ten plants, followed by cluster no. 6 with nine plants, cluster 5 contained 6 plants followed by cluster number 1 and 2 with 3 plants each and cluster number 4 with single plant.

The maximum distance between cluster centroids was observed between cluster 1 and 4 (7.642) followed by cluster 3 and 4 (7.449), cluster 4 and 5 (6.632). However, minimum distance was observed between cluster 2 and 6 (2.343). The average distance between the cluster members from cluster centroids indicated that plants in cluster 3 showed wide diversity (3.6) and the members of cluster 2 were found to be closely related (1.4) (Table 4.3).

The mean and standard deviation is presented in Table 4.4. The different characters studied are described below:

1. **Number of tillers:** Out of the four accessions of *P. glaucum*, T2 (or IG 2000-01) had the lowest value for number of tillers (1.3) while the highest was observed in 81A1 (7.0). In *P. violaceum*, the range of this character was between 2.7 (IP 21534) and 15.0 (IP 21532), in *P. schweinfurthii* it was recorded between 4.3 (PS 233) and 10.3 (IP 24214). In *P. polystachyon*, the lowest value was 17.0 (IP 22121) and the highest was recorded as 23.7 (IP 22109) similarly in *P. pedicellatum*, the lowest was recorded as 12.7 (NATP D-1) and highest was 41.7 (octoploid Agros 4). Considerable difference was observed in the range of number of tillers in various accessions of *P. ramosum*, *P. orientale*, *P. divisum*, *P. flassidum* and *P. squamulatum*.

When all the species were compared it was observed that the number of tillers ranged between 1.3 in *P. glaucum* (T2) to 110.7 in *P. divisum* (IP 21962). The coefficient of variation was the second highest for this character (34.9).

2. **Height of main tiller (cm):** In *P. glaucum*, the shortest height was 92.3 (81B) and the tallest was recorded as 140.0 (T2). The height of main tiller in *P. violaceum*, ranged from 148.3 (IP 21634) to 220.0 (IP 21534). In *P. schweinfurthii*, the value ranged between 158.3 (IP 24214) and 226.7 (PS 233). In *P. polystachyon* the value ranged from 150.0 (IP 22109) and 166.7 (IP 22102) and in *P. pedicellatum*, the range was between 115.00 (NATP D-1) and 152.6 (IP 21971). No difference was observed in *P. flassidum* accessions.

At interspecific level, the height of the main tiller ranged from 50 – 280. The maximum height was attained by *P. mollisimum* (IP 21782) and the minimum by *P. hoheneckeri* (IP 21954). The coefficient of variation was 17.9 for this character.

3. **Number of leaves per tiller:** In *P. glaucum*, number of leaves were observed between 6.33 (81B) to 10.7 (81A4). In *P. violaceum*, the value was between 9.0 (IP 21634) and 10.3 (IP 21532) the range was quite high in *P. schweinfurthii*, with 8.7 (IP 24214) and 12.0 (PS 233). *P. polystachyon*, showed variation between 7.3 (IP 22109) and 9.7 (IP 22121) and in *P. pedicellatum*, it ranged from 5.7 (Agros 4) to 8.3 (hexaploid IP 21971). Minor difference was observed in *P. squamulatum*, (10.7 and 10.3). Interspecific variation for number of leaves was found and the value was the lowest (5.7)

in two species *P. setaceum* (IP 21949) and *P. pedicellatum* (Agros 4) and highest (16.0) in the *P. setosum* (IP 21942). The coefficient of variation was 14.9.

4. Flag leaf length (cm): Very high intraspecific variation was observed in accession of *P. glaucum* between 11.7 (IG 99-748 or T1) and 42.7 (81B) and in *P. ramosum*, between 7.00 (IP 21935) and 78.3 (IP 22137). In *P. violaceum*, the variation was between 10.7 (IP 21634) and 25.0 (IP 21534). *P. schweinfurthii*, showed variation for this character between 8.7 (PS 233) to 21.3 (IP 21929). *P. polystachyon*, showed little variation from 5.3 (IP 22121) to 8.2 (IP 22102). In *P. pedicellatum*, the range was observed between 4.3 (NATP D-1) and 13.3 (IP 22095).

At interspecific level, the longest flag leaf (78.3) was observed in *P. ramosum* (IP 22137) and the shortest (4.3) in *P. pedicellatum* (NATP D-1). The coefficient of variation was found to be 20.4.

5. Flag leaf width (cm): The flag leaf width had lowest value (2.5) in the diploid accessions of *P. glaucum* while highest value (3.5) was observed in tetraploid (T1). The value of this character in *P. violaceum* ranged between 0.7 in IP 21634 and 2.1 in IP 21534. The range in *P. schweinfurthii* was observed between 1.2 (PS 233) and 1.8 (IP 21929). In *P. polystachyon* the value varied from 0.3 (IP 22121) to 0.6 (IP 22102). In *P. pedicellatum* the lowest value was recorded as 0.4 in the tetraploid accession (IP 22095) and the highest as 1.2 (NATP D-1). When accessions of all species were compared the lowest flag leaf width (0.3) was noted in *P. ramosum* (IP 21935) and *P. setaceum* (IP 21949) while highest value (3.5) for the character was observed in *P. glaucum* (T1). The coefficient of variation was 40.3.

6. 3rd leaf length (cm): The diploid male sterile accession of *P. glaucum* (81A1) had the shortest 3rd leaf (39.0) and tetraploid (T1) had the longest (71.7). In *P. violaceum* the lowest value 22.7 was observed in accession IP 21634 and the highest value was 38.3 in accession IP 21534. *P. schweinfurthii* (IP 21929) showed the shortest 3rd leaf growth (38.7) and IP 24214 had the longest leaf (48.3). *P. polystachyon* showed lowest variation between 13.5 (IP 22121) and 15.5 (IP 22102). *P. pedicellatum* showed variation between 17.3 (NATP D-1) and 22.5 (IP 21971)

The variation just like the flag leaf length was quite high at interspecific level with 10.5 to 71.7. The shortest 3rd leaf was found to be in *P. ramosum* (IP 21935) and the longest in *P. glaucum* (T1). The coefficient of variation was 13.6.

7. **3rd leaf width (cm):** The value of 3rd leaf width was found to vary between 2.7 (81B) and 4.0 (T2) in *P. glaucum*. In *P. violaceum*, the lowest value (1.1) was observed in accession IP 21634 and the highest (2.3) was in IP 21534. In *P. schweinfurthii*, not much variation was seen for the character and the value ranged from 2.2 (IP 24214) to 3.0

(PS 233). The lowest value in *P. polystachyon* was observed as 0.6 (IP 22109) and the highest was 1.1 (IP 22102). In *P. pedicellatum* the value varied between 0.9 in the tetraploid accession (IP 22095) and 1.3 in the hexaploid (IP 21971). At interspecific level the values for the character ranged from 0.4 to 4.0 with the lowest value in *P. villosum* (IP 21945) and the highest in the *P. glaucum* (T2). The coefficient of variation was found to be 17.8.

8. **Stem girth (cm):** Both the diploid accession of *P. glaucum* (81A1 and 81B) had the same and low value of stem girth (1.0) while the tetraploid (T1) had quite high value (1.7). Not much variation was observed in *P. violaceum* and *P. squamulatum* for the character. *P. schweinfurthii* showed variation between 0.8 (IP 21929) and 1.1 (PS 233). The value for the character in *P. polystachyon* ranged between 0.4 (IP 22159) and 0.5 (IP 22102) and in *P. pedicellatum* it ranged between 0.3 in tetraploid accession (IP 22095) and 0.7 in the hexaploid accession (IP 21971).

At interspecific level, the range of stem girth was highest in tetraploid *P. glaucum* (T1) with 1.7 and was recorded lowest in *P. villosum* (IP 21945) with 0.2. The coefficient of variation was 20.1.

9. **Number of nodes:** High variation for this character was observed in *P. glaucum* the diploid accession (81B) had the lowest value 4.7 while the other diploid accession (81A1) had the highest value 10.0. The tetraploid accessions had the intermediate values. In

P. violaceum, the lowest number of nodes (7.3) was observed in IP 21634 and the highest (9.7) in IP 21534. In *P. schweinfurthii*, the value ranged from 6.3 (IP 24214) to 9.7

(PS 233). In *P. polystachyon* the value varied from 7.3 (IP 22109) to 9.7(IP 22121). In *P. pedicellatum* the lowest value was recorded as 5.7 in the octoploid accession (Agros 4) and the highest as 8.3 in the hexaploid accession (IP 21971). The other species showed little intraspecific variation.

On comparing all the species it was observed that the number of nodes ranged from 4.0 to 11.7. The lowest number of nodes was observed in the *P. setosum* IP 21942 (4.0) while the highest (11.67) were observed in the *P. mollisimum* (IP 21782). The coefficient of variation for this character was observed to be 23.5.

10. Internode length (cm): The variation for the character was quite high in *P. glaucum.* The lowest value (11.0) was observed in the diploid accessions 81B while highest value (16.7) was observed in tetraploid T2. The value in *P. violaceum* ranged between 19.3 in IP 21534 and 22.0 in IP 21534. The range in *P. schweinfurthii* was observed between 15.0 (PS 233) and 23.2 (IP 24214). In *P. polystachyon,* the value varied from 17.5 (IP 22121) to 19.5 (IP 22102). In *P. pedicellatum* the octoploid accession (Agros 4) had the lowest value for the internode length (12.0) and the highest value 17.0 was observed in two accession viz. hexaploid NATP D-1 and tetraploid IP 22095. *P. flassidum* showed very little variation while the two accession of *P. squamulatum,* IG 98-360 and IG 2000-36 showed a high variation (16.0 and 29.0) for internode length. The internode length at interspecific level, ranged from 5.0 to 29.0. The lowest value was observed in the *P. orientale* IP 21951 (5.0) while the highest was observed in the *P. squamulatum* IG 2000-36 (29.0). The coefficient of variation for this character was observed 20.9.

11. Peduncle length (cm): The tetraploid accession (T1) of *P. glaucum* had the longest peduncle (38.3) while T2 had the shortest peduncle (25.0). Both the diploid accessions (81A1 and 81B) had the intermediate values. In *P. violaceum* the lowest value (13.5) was observed in accession IP 21634 and the highest (22.0) was in IP 21534. In *P. schweinfurthii,* the value ranged from 23.8 (IP 24214) to 37.7 (IP 21929). The shortest peduncle (17.7) in *P. polystachyon* was observed in accession IP 22102 and the longest (24.0) in accession IP 22121. In *P. pedicellatum,* the value varied between 11.7 in the hexaploid (IP 21971) and 18.7 in hexaploid (NATP D-1). Considerable difference was observed in the range of peduncle length of other accessions of the species. The interspecific variation was quite high with 10.3 to 57.0. The shortest peduncle was found in *P. ramosum* IP 22137 and the longest in *P. squamulatum* IG 98-360. The coefficient of variation was 19.2.

12. Spike length (cm): In *P. glaucum,* spike length showed little variation and the shortest spike (22.3 cm) was observed in the diploid accession (81B). The diploid accession 81A1 and the tetraploid accession T1 had the highest value with 25.7. In

P. violaceum, spike length ranged from 9.0 (IP 21634) to 14.7 (IP 21534). *P. schweinfurthii* (IP 24214) had the shortest spike with 17.5 length and PS 233 had the longest with 31.7 length. The range of spike length was observed between 13.7 (IP 22102) to 19.0 (IP 22121) in *P. polystachyon* and between 12.3 (NATP D-1) to 14.5 (IP 22095) in *P. pedicellatum.* High variation was observed in *P. squamulatum* accessions and no variation in *P. orientale.*The interspecific variation for spike length was from 6.3 to 31.7. The shortest spike was found in *P. ramosum* IP 22137 and the longest in *P schweinfurthii* (PS 233). The coefficient of variation was 15.3.

13. Spike width (cm): In *P. glaucum* spike width varied from 1.1 (T1) to 3.3 (81B). Little variation was observed in *P. violaceum.* The spike width was observed to be 1.3 in two accessions (IP 21634 and IP 21532) and 1.6 in one accession (IP 21534). In *P. schweinfurthii,* the value varied between 2.3 (PS 233) and 3.3 (IP 24214). *P. polystachyon* showed variation between 0.9 (IP 22121) and 1.5 (IP 22102). In *P. pedicellatum,* the range was from 1.3 (NATP D-1 and Agros 4) to 1.5 (IP 22095). No variation for this character was observed in *P. flassidum.*

The interspecific variation for spike width was from 0.7 to 3.3 with the lowest value in *P. flassidum* IP 22195 and the highest in the *P. glaucum* (T1). The coefficient of variation was found to be 20.4.

14. Number of florets per spikelet: *P. glaucum* showed no intraspecific variation for this trait. *P. violaceum* showed very little variation with 2.0 (IP 21532) to 3.3 (IP 21534). The variation was quite high in *P. schweinfurthii* and was observed between 2.0 (PS 233) and 7.3 (IP 24214). *P. ramosum, P. orientale, P. divisum* and *P. flassidum* showed no or very little variation for the trait. In *P. polystachyon,* the value varied from 1.0 (IP 22121) to 4.0 (IP 22102). In *P. pedicellatum* hexaploid (NATP D-1) had the lowest value 2.0 and the highest value 4.7 was observed in octoploid (Agros 4). *P. squamulatum* showed high variation for the trait. The number of florets per spikelet among all the species, ranged from 1.0 to 14.0. The lowest number of florets per spikelet were observed in *P. hoheneckeri* (IP 21954) while the highest were observed in the *P. squamulatum* IG 2000-36. The coefficient of variation for this character was observed to be 27.5.

Mean performance and variability (Table 4.5): Wide range of variability was observed for most of the characters like number of tillers (1.3 – 110.7), height of main tiller (50.0 -280 cm), flag leaf length (4.3 -78.3 cm) and width (0.3 -3.5 cm), 3rd leaf

length and width (10.5 -71.7 cm and 0.4 -4.0 cm respectively), internode length (5.0 - 29.0 cm), peduncle length (10.3 -57.0 cm), spike length and width (6.3 -31.7 cm and 0.7 -3.3 cm respectively) and number of florets per spikelet (1-14). The highest phenotypic coefficient of variation was observed in the case of number of tillers per plant (98.0). Relatively high phenotypic coefficient of variation was registered for several characters such as flag leaf width (87.6), flag leaf length (74.9), 3rd leaf width (72.6), number of florets per spikelet (70.1), stem girth (57.9), spike width (51.8), 3rd leaf length (49.6), peduncle length (47.7), height of main tiller (44.2), spike length (42.7), internode length (41), number of nodes (33.6). The number of leaves had the lowest phenotypic coefficient of variation (30.5).

Similarly the genotypic coefficient of variation was also highest for the number of tillers per plant (91.6) followed by flag leaf width (77.7), flag leaf length (72.1), 3rd leaf width (70.4), number of florets per spikelet (64.5), stem girth (54.4), 3rd leaf length (47.7), spike width (47.7), peduncle length (43.7), height of main tiller (40.4), spike length (39.8) Internode length (35.2), number of leaves (26.6). The number of nodes had the least genotypic variation (23.9).

Heritability and Genetic Advance: The heritability can be defined as the ratio of genotypic variance to total or phenotypic variance (broad sense) and the ratio of additive genetic variance to phenotypic variance (narrow sense). The heritability estimates in broad sense were obtained from analysis of variance from 14 metric traits and the results are given in the Table 4.5. The highest estimates of heritability (0.9) was recorded for 3rd leaf width, flag leaf length, 3rd leaf length, number of tillers, stem girth and spike length while moderate estimates of heritability (0.8) was recorded for height of main tiller, flag leaf width, peduncle length, spike width, number of florets per spikelet, number of leaves and internode length (0.7) and the lowest heritability was recorded for number of nodes (0.5)

Genetic advance is the improvement in mean genotypic value of selected plants over the parental population. The highest value of genetic advance was recorded for height of main tiller (104.3) and the lowest value of genetic advance was observed for stem girth (0.7). The moderate values were recorded for number of tillers (42.1) followed by 3rd leaf length (31.7), flag leaf length (28.2), peduncle length (19.57), spike length (12.6), internode length (9.59), number of floret per spikelet (4.3), number of

leaves (4.3), number of nodes (2.6), 3rd leaf width (1.9), flag leaf width (1.5) and spike width (1.4).

Correlation studies: The correlation studies were carried out among the 14 metric traits of the 32 *Pennisetum* accessions.

Genotypic correlation (Table 4.6): At the genotypic level, positive correlation was observed for the number of tillers with peduncle length (r=0.322). Negative correlation of number of tillers was observed with 3rd leaf length (r=-0.492), stem girth (r=-0.445), number of nodes (r=-0.422). Very high positive correlation was observed for height of main tiller with the internode length (r = 0.709) and number of nodes (r= 0.560). Positive correlation of height of main tiller was observed with 3rd leaf width (r=0.428) followed by spike length (r=0.425) number of leaves (r=0.400), 3rd leaf length, peduncle length, stem girth and flag leaf width. Number of leaves had a very high positive correlation with number of nodes (r=0.507). The highest positive correlation observed among all was of flag leaf width with 3rd leaf width (r=0.974) followed by stem girth (r=0.888) 3rd leaf length (r=0.673), spike length (r= 0.607) and spike width (r=0.503). However, positive correlation was obtained with peduncle length (r = 0.447) and number of florets per spikelet (r=0.356). High positive correlation of 3rd leaf length was obtained with peduncle length, spike length, 3rd leaf width, number of florets per spikelet and stem girth. Positive correlation was observed with spike width.

Very high positive correlation was observed for 3rd leaf width with stem girth, spike length and spike width. Positive correlation was observed with peduncle length. Very high positive correlation of stem girth was with spike length and positive correlation was observed with spike width. Peduncle length also had a very high positive correlation with spike length and number of florets per spikelet. Positive correlation of spike length was observed with spike width and number of florets per spikelet and between spike width and number of florets per spikelet.

Phenotypic correlation (Table 4.7): At the phenotypic level, number of tillers had a negative correlation with 3rd leaf width (r= - 0.442), stem girth (r=-0.373) and flag leaf width (r=-0.354). A very high positive correlation of height of main tiller was observed with internode length (r=0.627). Positive correlation of height of main tiller was with number of nodes (r = 0.409), 3rd leaf width (r=0.398), spike length (r=0.396), number of leaves, peduncle length, stem girth and 3rd leaf length. Flag leaf width showed the highest positive correlation with 3rd leaf width (r= 0.862) followed by stem girth, 3rd leaf

length and spike length and positive correlation with spike width and peduncle length. Similarly, 3rd leaf length had very high positive correlation with peduncle length, spike length and 3rd leaf width. Positive correlation was found with number of florets per spikelet, stem girth and spike width. A high positive correlation of 3rd leaf width was obtained with stem girth, spike length and spike width while positive correlation was observed with peduncle length. Stem girth had a positive correlation with spike length and spike width. Peduncle length had a high positive correlation with spike length and number of florets per spikelet. However, positive correlation of spike length was noted with stem girth and number of florets per spikelet respectively. A positive correlation of spike width was observed with number of florets per spikelet.

4.1.1.2. Variability for qualitative traits (Table 4.8, Plate 1):

1. Coloured ring at node: This character varied highly from green to dark violet in all the *Pennisetum* species. *P. glaucum* (81A1) had violet ring while the colour was observed above the nodes in the 81B, T1 had yellow coloured nodes while T2 had light violet nodes. All the three accessions of *P. violaceum* had different node colour (from yellow to dark violet). *P. mollisimum* had dark violet nodes. All accessions of *P. schweinfurthii* and *P. ramosum* showed green node colour and all accessions of *P. orientale* showed light violet colour while *P. divisum* showed yellow and violet nodes. Out of the two accessions of *P. flassidum,* one (IP 22195) showed yellow and the other one (IP 22200) showed violet colour above nodes. The various accessions of *P. polystachyon* and *P. pedicellatum* varied highly and *P. squamulatum* showed uniform node colour.

2. Node hairiness: The diploid accessions of *P. glaucum* (81A1 and 81B) were observed to have non hairy nodes while the tetraploids (Tetra 1 and Tetra 2) had hairy nodes. *P. violaceum, P. mollisimum* and *P. squamulatum* had hairy nodes. The character was partially exhibited in *P. orientale* and *P. divisum* while all the other species had non-hairy nodes.

3. Leaf base colour: Yellow leaf base colour was found to be predominant in most of the species. Out of the four *P. glaucum* accessions only 81A1 had light violet leaf base while the others had yellow leaf base. The three accessions of *P. violaceum* varied and had yellow, violet and dark violet leaf base. *P. schweinfurthii, P. ramosum, P. divisum, P. setaceum, P. hoheneckeri, P. setosum, P. villosum and P. squamulatum* were uniform

with yellow leaf base. The various accessions of *P. polystachyon* and *P. pedicellatum* varied and had yellow to dark violet leaf base.

4. Leaf hairiness: The hairiness of leaf was predominant in most of the species except *P. ramosum*. The character was partially exhibited in *P. schweinfurthii, P. orientale, P. divisum, P. flassidum, P. polystachyon, P. pedicellatum* and *P. squamulatum.*

5. Stigma colour: The white stigma colour was commonly found in *Pennisetum* species. But few species had violet, light violet or dark violet stigma. *P. orientale,* and *P. flassidum,* possess dark violet stigma colour while *P. divisum, P. polystachyon,* and *P. squamulatum* showed slight variation with violet, light violet or dark violet stigma. Stigma in *P. pedicellatum* showed white colour in three accessions (NATP D-1, IP 22095 and Agros 4) while IP 21971 showed violet stigma. *P. setaceum* showed violet stigma.

6. Stigma (bifid / trifid): All *Pennisetum* species showed bifid stigma except one accessions of *P. polystachyon* (IP 22102), and all accessions of *P. squamulatum,* in which both bifid and trifid stigma were observed.

7. Awn colour: The awn colour varied from yellow to dark violet. All accessions of *P. glaucum, P. ramosum* and *P. squamulatum* were observed to have yellow awn. Variation was observed in *P. violaceum* and *P. schweinfurthii* as two accessions of *P. violaceum* (IP 21634 and IP 21532) had yellow and single accession (IP 21534) had light violet awn. Similarly in *P. schweinfurthii,* two accessions (IP 21929 and PS 233) had dark violet while IP 24214 had yellow awns. The awns in *P. mollisimum, P. orientale* and *P. setaceum* were violet, whereas, in *P. divisum* and *P. flassidum,* awns were dark violet, and in *P. polystachyon* light violet or dark violet. Two accessions of *P. pedicellatum* showed yellow awns and two showed violet awns.

8. Life cycle: All the species belonging to primary gene pool, *P. schweinfurthii* and three accessions of *P. pedicellatum* (NATP D-1, IP 21971 and IP 22095) were observed to be annual, *P. ramosum* was observed to be biennial while all other species were perennial.

9. Habit: The habit in *Pennisetum* species was either erect or decumbent. The species, which showed decumbent habit, were *P. ramosum, P. divisum, P. flassidum, P. setosum* and *P. villosum.* All other species were of erect habit.

4.1.2. Cytological studies: To characterize the wild and cultivated *Pennisetum* species on the basis of cytology, meiotic studies of 27 accessions belonging to 13 species, were

carried out. To check the male fertility of the plants, pollen stainability test was also carried out (Table 4.9, Plate 2).

4.1.2.1. Species of primary gene pool:

1. *P. glaucum*

A. 81B: This was maintainer of male sterile lines with chromosome number 2n=2x=14. The study of 25 PMC showed the chromosomal configuration of 7_{II} at diakinesis of meiosis I. The average of ring bivalents was observed as 6.47 with a range of 5-7, rod bivalents were 0.5 with range 0-2. The chiasma frequency was 14. 17 and the range was 12-17.

Pollen fertility on the basis of pollen stainability was estimated as 93%. The mean pollen size was recorded 33.9 μm (range 24 μm to 39 μm).

B. 81A1: This was a male sterile line with 2n=2x=14. The chromosomal configuration of 7_{II} was recorded in the study of 30 pollen mother cells. The ring bivalents were present with an average of 6.3 and rod bivalents with 0.7. The range of ring bivalents was 5-7 and rod 0-2. As the plant was a sterile meiotic abnormalities like unequal distribution of chromosomes at anaphase I, formation of micro pollen were observed. The chiasma frequency per cell was observed 13.9 ranging from 11 to 16. Pollen grains analyzed by aceto-carmine stain were non-viable.

C. 81A4: This was also a male sterile line with 2n=2x=14 with A4 cytoplasm derived from *P. violaceum* (Marchais and Pernes 1985). It was maintained by *P. glaucum* (81B). The average chromosomal configuration of 7_{II} was recorded. The ring bivalents were present with an average of 6.6 and rod bivalents with 0.4. The range of ring and rod bivalents was 5-7 and 0-2, respectively. Meiotic abnormalities like unequal distribution of chromosomes at anaphase I, formation of micro pollen were observed. The chiasma frequency observed was 13.6 ranging from 12 to 14. The plant was male sterile. Pollen analyzed were shriveled unstained and unequal in size.

D. T1: This cytotype was a colchicine induced tetraploid of *P. glaucum* (81B). The plant exhibited average chromosomal association of $1.2_{I} + 7.16_{II} + 0.96_{III} + 2.4_{IV}$. The range was estimated as: univalents from 0 to3, bivalents from 3 to 12, trivalents from 0 to 4 and quadrivalents 0 to 5. The average of ring bivalents was 3.24 and range 0-8 while average of rod bivalents was 3.92 and range from 1 to 7. The most frequent association was $1_{I} + 4_{II} + 1_{III} + 4_{IV}$. Chiasma per cell ranged from 17-24 with a frequency of 19.64. Pollen stainability was 84%.

E. T2: Colchicine induced tetraploid of *P. glaucum* was procured from USDA with 2n=4x=28. Average chromosomal configuration was $0.35_{I} + 10.15_{II} + 0.25_{III} + 1.65_{IV}$. The ring bivalents were observed with an average of 6.3 ranging from 3 to10 and rod bivalents with 3.85 ranging from 0 to 6. Chiasma frequency was 22.4 and range was from 19-25 chiasma per pollen mother cell. The most frequent chromosomal association was $10_{II} + 2_{IV}$ with 40% occurrence followed by 15% with $12_{II} + 1_{IV}$ along with other associations. The pollen stainability was found to be 65%.

2. *P. violaceum*

A. PV 2433: This was diploid with 2n=2x=14. Meiotic studies showed average chromosomal configuration $0.1_{I} + 6.95_{II}$. Bivalents ranged from 6-7 while 0-2 univalents were observed in a few cells. The average of ring bivalents was 6 and range from 3-7 and that of rod bivalent was 0.95 and range from 0-4. The chiasma frequency was observed as 12.95 with a range from 10-14. The plant was male fertile. Pollen stainability was recorded as 98%.

B. IP 21532: This accession was diploid with 2n=2x=14 chromosome number. The study of 20 pollen mother cells showed similar results like accession PV 2433 of this species. Average chromosomal configuration was $0.1_{I} + 6.95_{II}$. Bivalents ranged from 6-7 while 2 univalents occurred exceptionally only in one pollen mother cell. Ring bivalents occurred with an average of 5.8 and rod bivalents with an average of 1.15. Range of ring and rod bivalents was 4-7 and 0-3, respectively. Chiasma frequency was found as 12.75 which was little lower than PV 2433. The chiasma per cell ranged from 11 to 14. This plant was male fertile with 98% pollen stainability.

C. IP 21586: The chromosome number of this plant was 2n=2x=14. 15 pollen mother cells were studied which showed perfect 7_{II} in all pollen mother cells. No univalents were observed. Ring bivalents observed at 6.86 average and rod at 0.1 ranging from 6-7 and 0-1, respectively. Chiasma frequency was found as 13.87 which was higher than *P. violaceum* (IP 21532) and little lower than PV 2433. The chiasma per cell ranged from 13 to 14. Pollen stainability was 98%.

3. *P. mollissimum* (IP 21782): This was a diploid species (2n=2x=14) belonging to the primary gene pool of pearl millet. Meiotic studies of 25 pollen mother cells showed that the average chromosomal configuration was $0.32_{I} + 6.84_{II}$. The univalents ranged from 0-4 and ring bivalents ranged from 4-6 while rod bivalents from 1-3. The average of ring

bivalents was 5.2 and that of rod bivalent was 1.6. Chiasma frequency was 12.04 with a range from 9-13. Pollen stainability was recorded as 77%.

4.1.2.2. Species of secondary gene pool:

4. *P. schweinfurthii* (PS 233): This species belong to the secondary gene pool of pearl millet. Chromosome number of this diploid species was 2n=14 with basic number x=7. Meiotic studies of 15 pollen mother cells revealed that the average chromosomal configuration was $0.26_{I} + 6.86_{II}$. The range of univalents was 0-2 and that of bivalents was 6-7. Ring bivalents were observed with a range from 1-3 with an average of 2.9 while rod bivalents ranged from 2-5 with an average of 3.9. The most frequent chromosomal association (86.7%) was 7_{II}. Up to 2 B chromosomes were observed in 26% pollen mother cells. Chiasma frequency was recorded as 9.8 with a range from 9-12. The plant exhibited 67% pollen stainability.

4.1.2.3. Species of tertiary gene pool:

5. *P. ramosum*

A. IP 22180: The accession exhibited 2n=2x=10 with basic chromosome number x=5 and is a member of tertiary gene pool. The average chromosomal association was $0.23_{I} + 4.88_{II}$. Bivalents ranged from 3-5. Chiasma frequency was 9.76 ranging from 6-10. Up to 2 B chromosomes were present in 58% cells. Pollen stainability was 99%

B. IP 22137: The accession exhibited 2n=2x=10 with basic chromosome number x=5. Perfect 5_{II} were observed in all 25 pollen mother cells studied. The average of ring bivalents was 4.9 and that of rod bivalents was 0.04. Chiasma frequency was 9.96 ranging from 9-10. The accession was male fertile with pollen stainability 96%.

6. *P. orientale* (IG 04-165): Meiotic studies revealed that this accession was a hexaploid with 2n=6x=54 chromosome number. 15 pollen mother cells showed average chromosomal configuration of $0.13_{I} + 22.6_{II} + 2.13_{IV}$. No trivalents were observed. 2 univalents were present only in one pollen mother cell. The range of bivalents was 19 to 26 while that of quadrivalents was 0 to 4. The chromosomal association $23_{II} + 2_{IV}$ observed in 40% cells. Chiasma frequency was 2.1 and range was from 0-4. The pollen stainability was found to be 85%.

7. *P. divisum* (IP 21962): The species is a tetraploid with 2n=36 and x=9. Total 25 pollen mother cells were studied which showed an average chromosomal configuration of $0.52_{I} + 15.8_{II} + 0.6_{III} + 0.52_{IV}$. Univalents ranged from 0-4, bivalents from 8-18, trivalents from 0-3 and quadrivalents from 0-3. Univalents were found in 16% pollen

mother cells. The average and range of ring bivalents was 13.5 and 5-18, and of rod bivalents was 2.3 and 0-10. The most frequent chromosomal association was 18_{II} (44%) followed by $16_{II} + 1_{IV}$ (24%) along with other associations. Chiasma frequency was 31.84 and range was from 24-36 chiasma per pollen mother cell. The pollen stainability was 86%.

8. *P. setaceum* (IP 21949): This was the only triploid species studied having 2n=3x=27. 25 pollen mother cells were studied. The average chromosomal configuration was found to be $7.16_{I} + 9.72_{II}$. Range of univalents was 3-15 and that of bivalents was 6-12. Ring bivalents were observed with an average of 8.9 and range 6-11 while rod bivalents were recorded with an average of 0.8 and range of 0-4. The most frequent chromosomal association was $7_{I} + 10_{II}$ (48%) followed by $9_{I} + 9_{II}$ (16%), $11_{I} + 8_{II}$ and $5_{I} + 11_{II}$ (12% each), along with other associations. Chiasma frequency per cell was estimated as 18.68 and range was 12-22. The pollen stainability was 24%.

9. *P. flassidum* (IP 22195): Cytological observations revealed that this was a pentaploid species with 2n=5x=45. The basic chromosome number was x=9. Meiotic studies of 25 pollen mother cells showed the average chromosomal configuration of $3.96_{I} + 17.48_{II} + 1.28_{III} + 0.56_{IV}$. The range of univalents was recorded 1-10, bivalents was 12-22, trivalents was 0-3 and quadrivalents ranged from 0-2. Ring bivalents were observed with an average of 10.44 while rod bivalents were recorded with an average of 7.04. Range of ring bivalents was 5-19 and rod with 2-14. The most frequent chromosomal association was $1_{I} + 19_{II} + 2_{III}$ with 16% occurrence. No pentavalents were observed. Chiasma frequency was 31.32 with a range of 24-41. Meiotic abnormalities were commonly observed. Lagging chromosomes up to 5, unequal distribution like 22+23 and 21+5+20 at anaphase I and micronuclei were observed. Pollen stainability was 47%.

10. *P. hoheneckeri* (IP 21954): This was a diploid accession with 2n=2x=18 which belongs to the tertiary gene pool of pearl millet. The average chromosomal configuration was $0.08_{I} + 8.96_{II}$. The most frequent chromosomal association was 9_{II} (96%). Equal distribution of 9+9 chromosomes was observed at anaphase I. Chiasma frequency was 15.32 and range was from 11-18 chiasma per cell. The pollen stainability was 65%.

11. *P. polystachyon* (IP 22102): A tertiary gene pool species with chromosome number 2n=6x=54. Total 20 pollen mother cells were studied which showed an average chromosomal configuration $1.8_{I} + 24.05_{II} + 0.3_{III} + 0.8_{IV}$. Univalents ranged from 0-8

while the range of bivalents was quite high from 20-27, trivalents from 0-6 and quadrivalents ranged from 0-2. Most frequent chromosomal association was 27_{II} (35%) followed by $25_{II} + 1_{IV}$ (15%) along with other associations. The pollen stainability was found to be 65%.

12. *P. pedicellatum*

A. NATP D-1: Meiotic studies showed that the chromosome number of this accession was 2n=6x=54. Study of 25 pollen mother cells showed the average chromosomal configuration was $2.2_{I} + 11.48_{II} + 1.88_{III} + 4.36_{IV} + 0.04_{V} + 0.88_{VI}$. Univalents ranged from 0-6, bivalents from 5-20, trivalents from 0-4, quadrivalents ranged from 2-7, pentavalent from 0-1 and hexavalent from 0-2. Frequently occurring chromosomal association was $10_{II} + 4_{III} + 4_{IV} + 1_{VI}$ with 8% occurrence. The pollen stainability was found to be 65%.

B. IP 21971: Meiotic studies showed that the chromosome number of this hexaploid species was 2n=6x=54. Study of 20 pollen mother cells showed the average chromosomal configuration of $0.1_{I} + 24.8_{II} + 0.75_{III} + 0.2_{IV} + 0.1_{V} + 0.15_{VI}$. The range of univalents was 0-1, bivalents from 18-27, trivalents from 0-4, quadrivalents ranged from 0-2, pentavalent from 0-1 and hexavalent from 0-3. No differentiation between ring and rod bivalents could be made as the chromosomes were very small. Frequently occurring chromosomal associations were 27_{II} (50%) followed by $24_{II} + 2_{III}$ (15%) and $25_{II} + 1_{IV}$ (10%). The pollen stainability was found as 63%.

C. IP 21790: This was a tertiary gene pool species with 2n=6x=54. Meiotic studies showed that the average chromosomal configuration in 20 pollen mother cells of this accession was $0.85_{I} + 13.75_{II} + 0.35_{III} + 4.2_{IV} + 1.3_{VI}$. The range of univalents was 0-4, bivalent was 10-20, trivalents was 0-2, quadrivalents ranged from 2-7 and hexavalent from 0-3. No pentavalents were observed. The most frequent chromosomal association was $11_{II} + 5_{IV} + 2_{VI}$ with 15%. Pollen stainability was 87%.

D. IP 21879: The chromosome number of this accession was 2n=6x=54. The average chromosomal configuration in 20 pollen mother cells of this hexaploid species was $1.05_{I} + 20.05_{II} + 0.75_{III} + 2.65_{IV}$. The range of univalents was 0-4, bivalent was 10-25, trivalents was 0-3, and quadrivalents ranged from 1-7. No pentavalents or hexavalents were observed. Only chain trivalents were observed. The most frequent chromosomal association was $25_{II} + 1_{IV}$ in 15%. Pollen stainability was 73%.

E. IP 22095: The only tetraploid accession of this species with 2n=4x=36. 25 pollen mother cells were cytologically analyzed which showed an average chromosomal configuration $0.08_{I} + 17.6_{II} + 0.24_{III}$. The range of bivalents was 15 to 18 and trivalents was 0-2. The most frequent chromosomal association was 18_{II} in 84%. The pollen stainability was 54%.

F. Agros 4: This was the only octoploid accession with 2n=8x=72 known so far. Meiotic studies showed that the average chromosomal configuration in 30 pollen mother cells was $1.03_{I} + 20.43_{II} + 3.47_{III} + 4.3_{IV} + 0.33_{V} + 0.1_{VI} + 0.03_{VII}$. The range of univalents was 0-5, bivalents was 12-25, trivalents was 1-6, quadrivalents ranged from 1-7 and pentavalents from 0-2, hexavalent from 0-2 and heptavalent 0-1. The most frequent chromosomal associations were $18_{II} + 4_{III} + 6_{IV}$, $25_{II} + 2_{III} + 4_{IV}$, $21_{II} + 2_{III} + 6_{IV}$ and $22_{II} + 4_{III} + 4_{IV}$ in 6.7% pollen mother cells. Pollen stainability was 69%.

13. *P. squamulatum*:

A. IG 98-360: In this accession ($2n=8x=56$), meiotic studies showed that the average chromosomal configuration in 20 pollen mother cells was $22.3_{II} + 0.25_{III} + 1.7_{IV} + 0.05_{V} + 0.6_{VI}$. The range of bivalents was 10 to 28, trivalents was 0 to 2, quadrivalents ranged from 0 to 6 and hexavalents from 0 to 2. Only one pentavalent was observed in only one pollen mother cell. Ring bivalents were observed with an average of 21.8 and range 10-28 while rod bivalents were recorded with an average of 0.5 and range 0-6. The most frequent chromosomal association was 28_{II} with 40%. Chiasma frequency was 53.3 with a range of 46-56. Meiotic abnormalities were observed. Early disjunction at metaphase I and up to 6 lagging chromosomes at anaphase I were observed. Bivalents were also found as laggards. Micronuclei were also observed. Pollen stainability was estimated as 71%.

B. IG 98-361 ($2n=8x=56$): Meiotic studies revealed that the average chromosomal configuration in 20 pollen mother cells was $2.85_{I} + 15.65_{II} + 1.7_{III} + 3.25_{IV} + 0.15_{V} + 0.5_{VI}$. Univalents ranged from 0-7, bivalents from 10-23, trivalents from 0-6, quadrivalents ranged from 1-6, pentavalent from 0-1 and hexavalents from 0-1. Pollen stainability was estimated as 71%.

C. IG 2000-36 Exotic material from USDA ($2n=8x=56$): Study of 20 pollen mother cells showed the average chromosomal configuration was $0.3_{I} + 19.8_{II} + 1.5_{III} + 2.9_{IV}$. Univalents ranged from 0-2, bivalents from 16-26, trivalents from 0-4, quadrivalents

ranged from 1-6. Frequently occurring chromosomal association was $21_{II} + 3_{IV}$ with 15%. The pollen stainability was found as 74%.

4.1.3. Biochemical studies:

4.1.3.1. Banding pattern of different isozymes in *Pennisetum* species: The study was undertaken in different accessions of *Pennisetum* species to estimate the genetic diversity among species. Four enzyme systems were studied i.e. EST, SOD, POD and GDH. POD was subjected to horizontal starch gel electrophoresis while the other three isozymes were subjected to polyacrylamide gel electrophoresis (PAGE) using discontinuous buffer system. 38 accessions of 16 *Pennisetum* species in EST and SOD, 41 accessions of 13 species in POD, and 24 accessions of 13 species in GDH were studied. To estimate the genetic diversity, the bands were scored and numbered on the basis of their relative mobility towards anodal/cathodal ends. A summary of nomenclature of bands with their respective Rm values is given in Table 4.10, Plate 3 represents these four enzyme systems.

4.1.3.1.1. Esterase (EST): The study showed total 26 bands (Table 4.11). The total number of bands in individual accessions ranged from 1 to 12. Band no. 24 (rm 0.72) was represented maximum times (63.16%) and the band no. 16 (rm 0.44) minimum times (2.63%). The open pollinated induced tetraploid of *P. schweinfurthii* (PS 233 a and b) exhibited single band (band no. 23, rm 0.7) and *P. setaceum* (IP 21949) exhibited the maximum of 12 bands.

Intraspecific variation was found in *P. orientale*, *P. polystachyon*, *P. divisum*, *P. hoheneckeri*, *P. pedicellatum*, *P. ramosum*, *P. violaceum* and *P. schweinfurthii*. No interspecific variation was found in *P. squamulatum* and *P. flassidum*. Variation was observed in *P. orientale* (IG 04-165) for band no. 17 (rm 0.46) and band 24 (rm 0.72) which were absent in other two accessions of *P. orientale* (IP 21951 and IP 22186). In the three accessions of *P. polystachyon*, only band no. 24 (rm 0.72) and band no.25 (rm 0.74) were similar. Out of the three accessions, *P. polystachyon* (IP 22121) showed maximum of ten bands. In *P. divisum* band no. 9 (rm 0.29), band no. 11 (rm 0.35), 13 (rm 0.41), 25 (rm 0.74) and band no. 26 (rm 0.76) were partly exhibited. Band no. 6 (rm 0.19) was species specific to *P. squamulatum* and band no. 1 (rm0.03) and 2 (rm 0.08) were specific to *P. ramosum*. Band no. 9 (rm 0.29), 11

(rm 0.35), 13 (rm 0.41), 25 (rm 0.74) and 26 (rm 0.76) were exhibited in *P. divisum*. Band no. 14 (rm 0.42) and 24 (rm 0.72) were exhibited in *P. ramosum*.

Intraspecific variation was observed in *P. hoheneckeri*. Band no. 11 (rm 0.35) and 12 (rm 0.36) were absent in IP 21954 while band no. 23 (rm 0.7) and 24 (rm 0.72) were absent in other two accessions IP 21953 and IP 21952. *P. setosum* (IP 21942) and *P. clendenstenum* (IG 04-166) differed only by one extra band (band no. 25, rm 0.74) in *P. clendenstenum*. In *P. pedicellatum* only two bands (band no. 24 and 25 with rm 0.72 and 0.74 respectively) were similar in all three accessions but variation was found for presence of band no. 14 (rm 0.42) and absence of band no. 23 (rm 0.7) which was present only in hexaploid *P. pedicellatum* (IP 21971), band no. 16 (rm 0.44) and 22 (rm 0.66) which were observed only in tetraploid *P. pedicellatum* (IP 22095). In *P. ramosum* band no. 14 (rm 0.42) was partly exhibited. Band no. 7 (rm 0.22) was specific to *P. squamulatum* and *P. violaceum*. Three additional bands were observed in *P. violaceum* (IP 21634) which were absent in other two accessions (IP 21532 and IP 21534). These are band no. 4 (rm 0.16), 24 (rm 0.72) and band no. 25 (rm 0.74). Band no. 10 (rm 0.32) was absent in *P. violaceum* (IP 21532). Two additional bands were observed in accession no. IP 21534. These were band no. 19 (rm 0.6) and band no. 20 (rm 0.62).

4.1.3.1.2. Superoxide desmutase (SOD): The study indicated the presence of total 9 bands. The banding pattern and rm values for individual band are given in Table 4.12. The total number of bands in accessions ranged from 1 to 4. One band was represented in *P. schweinfurthii* (all accessions) and P. *ramosum* (IP 21935). Band no. 1 (rm 0.38) and 7 (rm 0.88) was represented maximum times (77.14%) while band no. 2 (rm 0.54), 8 (rm 0.9) and band no. 9 (rm 0.92) occurred at (5.71%). Band no 8 and 9 were specific to *P. squamulatum*. Band no. 1 (rm 0.38) was represented in all species except *P. schweinfurthii* whereas partly exhibited in accessions of *P. ramosum* (IP 22137) and *P. hoheneckeri* (IP 21953 and IP 21952).

P. orientale hexaploid accession (IG 04-165) had 2 extra bands (band no. 5, rm 0.79 and band no. 6, rm 0.84) which were absent in tetraploid accessions of *P. orientale* (IP 21951 and IP 22186). Band no. 1 (rm 0.38) and 7 (rm 0.88) were observed in all the three accessions of *P. orientale*. Similar results have been observed in *P. polystachyon*. One accession of *P. polystachyon* (IP 22102) lacks band no. 3 (rm 0.71) and 4 (rm 0.76) while other two accessions IP 22109 and IP 22121 exhibited total of four

bands i.e. band no. 1 (rm 0.38), 3 (rm 0.71), 4 (rm 0.76) and 5 (rm 0.79). The three accessions of *P. hoheneckeri* exhibited slight difference of only one band. No intraspecific variation was found in *P. squamulatum* (IG 98-360 and IG 98-361), *P. divisum* (IP 21957, IP 21963), *P. flassidum* (IP 22195, IP 22200, IP 22186), *P. violaceum* (IP 21634, IP 21532, IP 21534) and *P. schweinfurthii* (IP 21929, IP 21214, PS 233 diploid and induced tetraploid). Intraspecific variation was noted in *P. orientale* as accession IG 04-165 exhibited two extra bands band no. 4 and 5 along with the two bands 1 and 7 exhibited in the other two accessions.

Similarly accession IP 22102 of *P. polystachyon* also showed intraspecific variation as two bands (band no. 3 and 4) were absent while the other two accessions (IP 22109 and IP 22121) exhibited four bands, band no. 1, 3, 4 and 5.

P. hoheneckeri (IP 21954) differs from other two accessions (IP 21953 and IP 21952) in having band no. 2 instead of band no. 1, band no. 7 was exhibited in all the three accessions. Tetraploid *P. pedicellatum* (IP 22095) exhibited only band no. 1 and 5 instead of four bands 1, 3, 4, and 5 as in hexaploid (IP 21971) and NATP D-1 (Table 4.12).

4.1.3.1.3. Peroxidase (POD): The study showed the presence of 17 anodal and 10 cathodal bands. The rm of anodal bands ranged from 0.02 to 0.92 (Table 4.13a) whereas the cathodal bands ranged from 0.02 to 0.87 rm (Table 4.13b). The number of anodal bands ranged from 2 to 10 while cathodal bands ranged from 0 to 6.

Intraspecific variation was recorded in *P. violaceum* for three anodal bands i.e. band no. 2 (rm 0.03), 3 (rm 0.08) and 14 (rm 0.73) and 3 cathodal bands i.e. 1 (rm 0.02), 7 (rm 0.5) and 10 (rm 0.87). In anodal bands, band no. 16 (rm 0.83) was represented maximum times (53.66%) and the band no. 1 (rm 0.02) was represented minimum times (2.44%). At cathodal end, band no. 10 (rm 0.87) was represented maximum times with 63.41% and band no. 3 (rm 0.12) was represented minimum times with 9.76%. Diversity was also observed in the two diploid (81A and 81B) and two tetraploid (T1 and T2) accessions of pearl millet. The four accessions of *P. glaucum* share similarity in having anodal bands no. 11 (rm 0.48), 15 (rm 0.8) and cathodal band no. 10 (rm 0.87). *P. schweinfurthii*, accession no. PS 237 had a specific anodal band no. 1 (rm 0.02) with 2.44%. Anodal band no. 6 (rm 0.25) exhibited only in accession no. IP 21929. Band no. 5 (rm 0.21) was present in 50% of *P. schweinfurthii* accessions. The

four accessions were similar in having anodal band no. 3 (rm 0.08), 4 (rm 0.12) and cathodal band no. 10 (rm 0.87).

The three accessions of *P. ramosum* had diversity only for two bands. Anodal band no. 8 (rm 0.4) that was observed in accession no. IP 22180 and cathodal band no. 9 (rm 0.8) in IP 22137. *P. squamulatum* showed intraspecific similarity in having anodal band no. 4 (rm 0.12) and 14 (rm 0.73) and cathodal band no. 5 (rm 0.25) and 10 (rm 0.87) in all accessions. Anodal band no. 17 (rm 0.92) was specific to two plants viz. *P. squamulatum* IG 2000-36 and *P. polystachyon* IP 21902. Intraspecific variation was noted in the two accessions of *P. orientale* as anodal band 5 (rm 0.21), 6 (rm 0.25) and 16 (rm 0.83) and cathodal band no. 6 (rm 0.42) and 7 (rm 0.5) were partly exhibited. All accessions of *P. pedicellatum* had only one similar anodal band no. 16 (rm 0.83) while five (83.3%) similar bands were observed in the *P. polystachyon* accessions.

4.1.3.1.4. Glutamate dehydrogenase (GDH): The study showed the presence of only two bands in 24 accessions of *Pennisetum* species studied. Band no. 2 (rm 0.09) appeared only in two accessions (*P. clendenstenum* IG 04-166 and *P. setosum* IP 21942) and band no. 1 (rm 0.04) appeared in all other species studied (Table 4.14).

4.1.3.2. Similarity among different *Pennisetum* species on the basis of different enzymes: The similarity between the plants was estimated using the Jaccard similarity matrix. Dendogram was formed by SAHN agglomerative clustering using the UPGMA algorithm.

4.1.3.2.1. Esterase (EST): The dendogram based on 26 bands of Esterase enzyme system of thirty eight plants showed the presence of 8 main clusters which were further divided in total 20 sub-clusters (Fig. 4).

Cluster –1 comprised of only one accession of *P. schweinfurthii* that was totally different from the other plants. No similarity of this plant with other species was observed.

Cluster –2 comprised of accession of *P. schweinfurthii* that showed 100% similarity.

Cluster –3 comprised of four accessions. The cluster was further divided into 3 sub-clusters. Cluster 3-1 comprised of only *P. mollisimum* (IP 21782). Sub-cluster 3-2 comprised of two accessions *P. violaceum* (IP 21534 and IP 21532) that showed 50% similarity among themselves. Third sub-cluster 3-3 had one accession of *P. violaceum* (IP 21634) that share 45% similarity with 3-1 and 40% similarity with 3-2.

Cluster –4 comprised of two accessions of *P. hoheneckeri* (IP 21953 and IP 21952), that share 100% similarity among themselves.

Cluster –5 comprised of two accessions of *P. ramosum* that showed 67% similarity with each other. This was the bridging cluster between the clusters 1, 2, 3 and clusters 6, 7, 8.

Cluster –6 comprised of two sub-clusters with 42% similarity between the two sub-clusters. Sub-cluster 6-1 comprised of both the accessions of *P. glaucum* that showed 57% similarity. Sub-cluster 6-2 contained all the three accessions of *P. orientale*. The two sub-clusters shared 43% similarity.

Cluster –7 comprised of ten accessions further divided into 3 sub-clusters. The sub-cluster 7-1 comprised of two accessions of *P. schweinfurthii* (IP 24214 and PS 233), single accession of *P. polystachyon* and *P. pedicellatum* each. The sub-cluster 7-2 comprised of *P. hoheneckeri* (IP 21954) and *P. setosum* (IP 21942) accessions that share 68% similarity. Four species were grouped in sub-cluster 7-3.

Cluster –8 was similar in size with cluster 7 and comprised of ten plants, and three sub-clusters. Sub-cluster 8-1 with 2 plants, 8-2 with 3 plants (all with 100% similarity) and 8-3 with five plants these three sub-clusters share similarity of 30% and 35% respectively.

Grouping of species on the basis of EST enzymes among 38 accessions of *Pennisetum*:

Cluster no.	Sub-cluster no.	Sample/plant no.*	Species/Accessions
1	1-1	33	*P. schweinfurthii* PS 233 (4x self)
2	2-1	34, 35	*P. schweinfurthii* PS 233 (4x OP) a, b
3	3-1	29	*P. mollisimum* IP 21782
	3-2	27, 28	*P. violaceum* IP 21532, IP 21534
	3-3	26	*P. violaceum* IP 21634
4	4-1	17, 18	*P. hoheneckeri* IP 21953, IP 21952
5	5-1	24, 25	*P. ramosum* IP 22137, IP 21935
6	6-1	36, 37	*P. glaucum* IG 99-748, IG 2000-01
	6-2	2, 3, 4	*P. orientale* IP 21951, IP 22186, IG 04-165
7	7-1	22, 31	*P. pedicellatum* IP 21971(6x), *P. schweinfurthii* IP 24214
	7-2	7, 32	*P. polystachyon* IP 22109, *P. schweinfurthii* PS 233 (2x X)
	7-3	16, 19	*P. hoheneckeri* IP 21954, *P .setosum* IP 21942
	7-4	6, 20, 21, 23	*P. polystachyon* IP 22102, *P. clendenstenum* IG 04-166, *P. pedicellatum* (NATP D-1), IP 22095(4x)
8	8-1	10, 30	*P. divisum* IP 21963, *P. schweinfurthii* IP 21929
	8-2	13, 14, 15	*P. flassidum* IP 22195, IP 22200, IP 22188
	8-3	5, 8, 12, 38, 1	*P. setaceum* IP 21949, *P. polystachyon* IP 22121, *P. squamulatum* IG 98-360, IG 98-361, IG 2000-36

* Ref table 4.11.

4.1.3.2.2. Superoxide Desmutase (SOD): The dendogram based on 9 bands of SOD enzyme system of thirty eight plants showed the presence of 5 main clusters which were further divided in total 8 sub-clusters (Fig. 5).

Cluster –1 comprised of 5 accessions (2 of *P. polystachyon* IP 22109, IP 22121, two accessions of *P. pedicellatum* IP 21971, NATP D-1 and *P. clendenstenum* IG 04-166) all belonging to tertiary gene pool and showed 100% similarity.

Cluster –2 comprised of 2 sub-clusters with 3 plants in sub-cluster 2-1 and 5 in 2-2. All accessions of this cluster belong to tertiary gene pool. The intra-cluster similarity was found to be 50%. Sub-cluster 2-1 comprised of three different species (*P. polystachyon* IP 22102, *P. setosum* IP 21942 and *P. pedicellatum* IP 22095). Sub-cluster 2-2 comprised of one *P orientale* accession (IG 04-165), one *P. villosum* (IP 21945) and all the three accessions of *P. flassidum.*

Cluster –3 comprised of eight plants, which were further divided into two sub-clusters. Subcluster 3-1 was represented by all the six accessions of *P. schweinfurthii* and one accession of *P. ramosum* (IP 21935) and 3-2 was represented by a single *P hoheneckeri* accession (IP 21954). The two sub-clusters showed 50% similarity.

Cluster –4 was the largest among all the clusters with 14 plants. The sub-clusters 4-1 with only one accession (*P. orientale* IG 04-165) and 4-2 with 13 accessions (all accessions of *P. violaceum*, two accessions of *P .orientale*, both accessions of *P. divisum*,

P. hoheneckeri and *P. glaucum* each, *P. ramosum* IP 22137, and *P. mollisimum*). The two sub-clusters had 66% similarity.

Cluster –5 was the smallest cluster that comprised of all the three accessions of *P. squamulatum.*

Grouping of species on the basis of SOD enzymes among 38 accessions of *Pennisetum*:

Cluster no.	Sub-cluster no.	Sample/plant no.*	Species/Accessions
1	1-1	7, 8, 20, 21, 22	*P. polystachyon* IP 22109, IP 22121, *P. clendenstenum* IG 04-166, *P. pedicellatum* (NATP D-1), IP 21971(6x)
2	2-1	6, 19, 23	*P. polystachyon* IP 22102, *P. setosum* IP 21942, *P. pedicellatum* IP 22095 (4x)
	2-2	4, 11, 13, 14, 15	*P. orientale* IG 04-165, *P. villosum* IP 21945, *P. flassidum* IP 22195, IP 22200, IP 22188
3	3-1	25, 30, 31, 32, 33, 34, 35	*P. ramosum* IP 21935, *P. schweinfurthii* IP 21929, IP 24214, PS 233 (2x), PS 233 (4x self), PS 233 (4x OP) a, PS 233 (4x OP) b
	3-2	16	*P. hoheneckeri* IP 21954
4	4-1	5	*P. setaceum* IP 21949
	4-2	2, 3, 9, 10, 17, 18, 24, 26, 27, 28, 29, 36, 37	*P. orientale* IP 21951, IP 22186, *P. divisum* IP 21957, IP 21963, *P. hoheneckeri* IP 21953, IP 21952, *P. ramosum* IP 22137, *P. violaceum* IP 21634, IP 21532, IP 21534, *P. mollisimum* IP 21782, *P. glaucum* IG 99-748, IG 2000-01
5	5-1	1, 12, 38	*P. squamulatum* IG 2000-36, IG 98-360, IG 98-361

* Ref table 4.12.

4.1.3.2.3. Peroxidase (POD): The dendogram based on 17 anodal bands and 10 cathodal bands of forty one accessions showed the presence of 5 main clusters which were further divided in total 9 sub-clusters (Fig. 6).

Cluster –1 comprised of 3 *P. pedicellatum* accessions (IP 21879, IP 21790, IP 21971(6x)) out of which 32 and 33 share 100% similarity while these two share 75% similarity with 35.

Cluster –2 comprised of 13 plants and 5 sub-clusters that showed intra sub-cluster similarity ranging from 0.49 to 100%. Cluster 2-1 comprised of two plants of distinct tertiary gene pool species (*P. divisum* IP 21962 and *P. pedicellatum* IP 22095). Sub-cluster 2-2 comprised of three accessions (*P. pedicellatum* Agros 4, IP 21890, IP 21883). Cluster 2-3 comprised of single accession of *P. squamulatum* (IG 2000-36). All *P. polystachyon* accessions and single *P. pedicellatum* accession (NATP D-1) were clustered in sub-cluster 2-4. Single accession of *P. glaucum* (T1) was clustered in sub-cluster 2-5.

Cluster –3 was divided in three sub-clusters that comprised of seven accessions. Out of these seven accessions, six belongs to tertiary gene pool and one (*P. mollisimum*) belongs to primary gene pool. The tertiary gene pool species includes two *P. ramosum* and all *P. orientale* accessions and one accession of *P. setaceum* (IP 21949 IPK). Sub-cluster 3-1 comprised of three plants *P. setaceum* (IP 21949 IPK) and *P. orientale* (IG 04-165, IP 21951). Sub-cluster 3-2 also comprised of three accessions, one of *P. mollisimum* (IP 21782) and two of *P. ramosum* (IP 22180, IP 21935) and sub-cluster 3-3 comprised of single accession of *P. schweinfurthii* (PS 237).

Cluster –4 comprised of three *P. schweinfurthii* accessions that share 76% to 86% similarity and single unidentified species. This cluster appeared to be a bridge between the two clusters 3 and 5

Cluster –5 was the largest among all the clusters and comprised of 14 accessions that showed intra-cluster similarity between 50% and 100%. The cluster was further sub-divided into 3 sub- clusters. Sub-cluster 5-1 was represented by a single *P. setaceum* accession (IP 21949). Sub-cluster 5-2 was represented by two accessions, one of *P. ramosum* (IP 22137) and one of *P. squamulatum* (IG 98-360). Sub-cluster 5-3 comprised of 10 accessions. Out of these, two species (*P. glaucum* and *P. violaceum*)

were of primary gene pool and 2 tertiary gene pool species (*P. flassidum* IP 22195 and *P. squamulatum*).

Grouping of species on the basis of Peroxidase enzymes among 41 accessions of *Pennisetum*:

Cluster no.	Sub-cluster no.	Sample/plant no.*	Species/Accessions
1	1-1	32, 33, 35	*P. pedicellatum* IP 21879, IP 21790, IP 21971(6x)
2	2-1	21, 34	*P. divisum* IP 21962, *P. pedicellatum* IP 22095(4x)
	2-2	29, 31, 30	*P. pedicellatum* Agros 4, IP 21890, IP 21883
	2-3	24	*P. squamulatum* IG 2000-36
	2-4	36, 37, 38, 39, 40, 41	*P. polystachyon* IP 21900, IP 21904, IP 22121, IP 22102, *P. pedicellatum* NATP D-1, *P. polystachyon* IP 21902
	2-5	11	*P glaucum* IG 99-748
3	3-1	26, 27, 28	*P. setaceum* IP 21949 IPK, *P. orientale* IG 04-165, IP 21951
	3-2	17, 18, 19	*P. mollisimum* IP 21782, *P. ramosum* IP 22180, IP 21935
	3-3	16	*P. schweinfurthii* PS 237
4	4-1	12, 14, 13, 15	Unidentified species, *P. schweinfurthii* IP 21931, PS 2116, IP 21929
5	5-1	25	*P. setaceum* IP 21949
	5-2	20, 23	*P. ramosum* IP 22137, *P. squamulatum* IG 98-360
	5-3	1, 4, 3, 2, 6, 9, 10, 7, 22, 5, 8	*P. squamulatum* IG 98-361, *P. violaceum* IP 21524, IP 21634, IP 21579, IP 21634, *P glaucum* 81B, IG 2000-01, *P. violaceum* PV 2433, *P. flassidum* IP 22195, *P. violaceum* IP 21532, *P glaucum* 81A

* Ref table 4.13a and 4.13b.

4.1.3.2.4. Glutamate Dehydrogenase (GDH): The dendogram based on 2 bands of twenty four accessions showed the presence of 2 clusters (Figure 7).

Cluster -1 comprised of two accessions 15 and 24 (*P. clendenstenum* IG 04-166 and *P. setosum* IP 21942) which share 100% similarity with each other.

Cluster -2 comprised of 22 accessions that also share 100% similarity with each other.

Grouping of species on the basis of GDH enzymes among 41 accessions of *Pennisetum*:

Cluster no.	Sub-cluster no.	Sample/plant no.*	Species/Accessions
1	1-1	15, 24	*P. clendenstenum* IG 04-166, *P. setosum* IP 21942
2	2-1	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 16, 17, 18, 19, 20, 21, 22, 23	*P. squamulatum* IG 98-360, *P. violaceum* IP 21634, IP 21532, *P. mollisimum* IP 21782, *P. glaucum* IG 99-748, IG 2000-01, *P. schweinfurthii* 4x (x), 4x (OP) a, b, *P. squamulatum* IG 98-361, *P. flassidum* IP 22195, IP 22188, *P. hoheneckeri* IP 21953, IP 21952, *P. pedicellatum* NATP D-1, *P. polystachyon* IP 22102, IP 21971 (6x), IP 22095 (4x), *P. squamulatum* IG 2000-36, *P. setaceum* IP 21949, *P. orientale* IP 21951, IP 22186

* Ref table 4.14.

4.2. Induction of polyploidy:

Four diploid *Pennisetum* species (*P. violaceum, P. ramosum, P. schweinfurthii* and male sterile lines of *P. glaucum*) and tetraploid pearl millet were given colchicine treatment for induction of tetraploidy and octoploidy respectively. The

treatments were given to the seeds, seedlings, and to the tillers of the species. Colchicine was used at different concentrations of 0.05%, 0.1% and 0.2% and durations 6 hrs, 12 hrs, 18 hrs and 24 hrs (Table 4.15). Four types of colchicine treatments were given for induction of tetraploidy viz. seed treatment, shoot treatment, seedling capillary treatment, colchicine injection. The details of these treatments are described in section 3.2.3.

The seed treatment and the colchicine injection treatment did not produce any induced colchiploid plants. The colchicine + 2-4D injection was given to first internode (just below the peduncle) of the flowering tillers of 10 plants. The seeds thus produced were grown in the field and the plants were analyzed with flow cytometry to confirm the induction of colchiploidy. No induced colchiploid or any variant was observed.

Out of all these treatments only two treatments were successful. The seedling capillary treatment (treatment no. 3) was successful in the case of *P. schweinfurthii* and one tetraploid plant was observed. Shoot treatment (treatment no. 2) was successful in the case of diploid male sterile lines of *P. glaucum.* No induced tetraploid were observed in any accessions of other species (*P. violaceum* and *P. ramosum*) treated with colchicine. The induced tetraploids thus observed are discussed below:

1. *P. schweinfurthii* (PS 233): Out of all the treatments, only the capillary treatment (treatment no. 3) was successful in the case of *P. schweinfurthii* and one tetraploid plant was recovered. Out of the various capillary treatments at different concentrations of colchicine, 0.1% colchicine for 24 hour yielded three variants. Of which, two plants were found to be vigorous with enhanced leaf size, whereas one plant was more or less similar to diploid parent.

Cytological analysis of the two vigorous plants revealed them to be diploids with 2n=14 chromosomes, but their pollen stainability was reduced to 67.5% and 49.6% respectively as compared to 96% of normal diploid control. The cytological analysis of the third plant revealed it to be a tetraploid with 2n=(4x)=28 chromosomes, as compared to 2n=2x=14 chromosomes of the diploid parent. Average chromosomal association per cell was observed to be 0.95_{I} $+10.3_{II}$ $+0.35_{III}$ $+1.35_{IV}$. The association in diploid being 0.26_{I} $+6.86_{II}$ (Table 4.16 Plate 4). The chiasmata frequency was recorded as 17.55 and range was 13-25 in the tetraploid while that of diploid was 9.8 and

9-12 respectively. Pollen stainability in tetraploid was reduced to 80% from 96% in the diploid. The spikes of this plant were self-pollinated to produce seeds. These seeds (C_1 generation) were grown in pots and were allowed to grow. On flowering the spikes were cytologically analyzed and it was observed that the plant reverted to diploid condition with 2n=14 chromosome number.

2. *P. glaucum*: The colchicine treatment to shoot (treatment no. 2) was successful in the case of two male sterile lines of *P. glaucum* and two tetraploid plants were recovered (Plate 5). The treatment was given to 150 plants in the month of January. On flowering, these plants were cytologically analyzed and out of the various treatments at different concentrations of colchicine, 0.2% colchicine for 24 hour yielded two tetraploid plants, viz. one 81A1 and one 81A4. No induced tetraploid was recovered in case of 81A5 plants. The two induced tetraploid plants from 81A1 and 81A4 were found to be vigorous with enhanced stomata and leaf size. Induction of tetraploidy was confirmed by flow cytometry (Figure 2). These two induced tetraploids are discussed below:

81A1: The colchicine treated (C_0 generation) plant was morphologically less vigorous and smaller than the diploid one. The metric trait, measurements were less than the diploid plant for all the characters (Table 4.17). Qualitative characters showed little difference in both the plants (Table 4.18). The young leaves of this plant were taken for flow-cytometric analysis which confirmed it to be a tetraploid as the DNA content of this plant was just double than the diploid plant. The impression of stomata was taken by applying thin film of quickfix on the leaves of both control (diploid) and the treated plants. The induced tetra had larger stomata (14.6 µm) than the diploid one (8.5 µm) and the number of stomata in the induced tetraploid and the diploid ranged between 3-5 per microscopic field and 7-8 per microscopic field, respectively (Plate 5, picture16 and 17). The tetraploid nature of this plant was further confirmed by cytological analysis. It contained 2n=4x=28 chromosome number as compared to 2n=2x=14 chromosomes of the diploid parent. Average chromosomal association per cell at diakinesis was observed to be $0.8_{I} + 11.05_{II} + 0.7_{III} + 0.75_{IV}$. The association in the diploid parent being 7_{II}. The chiasmata frequency and range was observed to be 20.35 and 16-24 while in the diploid, it was 13.9 and 11-16 (Table 4.19). The unequal distribution of chromosomes at anaphase I was also commonly observed in the induced tetraploid. It was male sterile like the diploid parent. The pollen stainability was nil in these plants when stained with aceto-glycerocarmine and the pollen were shriveled and irregular in shape (Plate 6,

picture11 and 12). The pollen abortion was taking place at an early stage of microsporogenesis before the emergence of gynoecium.

The spikes of this plant were cross-pollinated with the pollen of T1 (tetraploid pearl millet). Numerous seeds were produced, both shrivelled and healthy. The seeds were sown in the pots and healthy plants were grown. On analyzing by flow-cytometer, these C_1 generation plants were observed to be tetraploids. The C_1 generation was again crossed with T1 and numerous seeds were obtained.

81A4: Tetraploid of this plant (C_0) was also morphologically less vigorous and short highted (60 cm) than the diploid plant (119.67 cm) (Table 4.17 and 4.18). The nature of the plant was analyzed by flow-cytometry and it was observed that the plant was a tetraploid. Cytological analysis revealed the chromosome number to be 2n=(4x)=28. This plant also contained larger stomata (14.4 μm) than the diploid one (9.6 μm) and the number of stomata varied from 3-5 per microscopic field in the tetraploid and 5-6 per microscopic field in the diploid. Average chromosomal association at diakinesis was observed to be $0.04_{I} + 11.16_{II} + 0.12_{III} + 1.32_{IV}$. The association in the diploid parent being 7_{II} (Table 4.19). The chiasmata frequency and range was observed to be 25.04 and 18-32 while in the diploid, it was 13.6 and 12-14. The unequal distribution of chromosomes at anaphase I was also observed in the induced tetraploid. The plant turned out to be a male sterile like the diploid parents. The pollen stainability was nil when stained with aceto-glycerocarmine and the pollen were shrivelled and irregular in shape (Plate 6). The pollen abortion in this plant was also taking place at an early stage of microsporogenesis before the emergence of gynoecium.

The spikes of this plant were also cross-pollinated with the pollen of T1. Numerous healthy seeds were produced (about 50 seeds per spike were produced). About 10 seeds were sown in the pots and healthy plants were grown. On analyzing by flow- cytometer, these C_1 generation plants were also observed to be tetraploids. When these C_1 plants were compared with T1 (tetraploid *P. glaucum*) and the diploid parent for morphological traits, it was found that the C_1 plants were highly vigorous for metric traits like height of main tiller and stem girth, than the diploid parent. Qualitative characters showed very little difference. The C_1 generation was again crossed with T1 and numerous seeds were formed. The pollen stainability was nil. The pollen stainability was nil however some plants were found to be pollen shedder. Irregularities in sterility behavior were observed when crossed with T1.

4.3. Interspecific hybridization:

The wild *Pennisetum* species were used in the interspecific hybridization (described in section 3.1.4 of material and methods).

Suitable accessions belonging to *Pennisetum* species with x=5, 7 and 9, such as *P. ramosum, P. schweinfurthii, P. squamulatum, P. orientale, P. pedicellatum, P. polystachyon, P. flassidum, P. orientale, P. setaceum* were selected for attempting crosses. Diploid male sterile lines and tetraploid lines of pearl millet were used for attempting crosses with diploid/tetraploid species. For polyploid species, tetraploid pearl millet lines were used. Diploid species were also crossed with tetraploid pearl millet and vice versa. 485 interspecific crosses were attempted in the present study (Table 4.20).

4.3.1. Embryo rescue (Table 4.21, Plate 7):

For embryo rescue, interspecific hybridization was carried out in the month of October and November of 2004. Two accessions of tetraploid pearl millet (T1 and T2) were used as female and two wild species *P. polystachyon* and *P. pedicellatum* were used as male parent. Two accessions of *P. polystachyon* (IP 22102 and IP 22109) and two accessions of *P. pedicellatum* (IP 22095 and octoploid) were used. These wild species does not cross with diploid pearl millet therefore, tetraploid pearl millet was used. By crossing with tetraploid pearl millet only shriveled seeds were formed. It was noticed that embryo abortion was taking place after 18-20 days of pollination. On the 17^{th} day, the healthy embryos were observed that started shriveling after 18^{th} day of pollination. To save the embryo, embryo rescue was attempted on 17^{th} day of pollination. The immature embryos were dissected out under aseptic conditions, sterilized with mercuric chloride and put in the culture media. Two culture media were used; ½ MS_0 and embryo culture media (ECM).

T1 x *P. polystachyon*: One hundred and fifty embryos of accession IP 22109 and fifty embryos of accession IP 22102 were attempted rescued. Germination took place on 4^{th} day in the control embryos (T1 and T2) and 5-7 days in crossed embryos. Some crossed embryos showed early germination while some of them germinated very late even after one week. The early germinated embryos were thought to be the self-pollinated one (the female being self compatible and selfing could be possible) and the embryos that showed late germination were expected to be the cross-pollinated ones. The slow and stunted growth and the morphology of the plantlets of these late germinated embryos showed

that these embryos were resultant from cross. These plantlets were vigorous than both the parents.

T1 x *P. pedicellatum*: Two accessions of *P. pedicellatum* were used in the crossing and embryo rescue (IP 22095 and Agros 4). IP 22095 was the tetraploid and the other one was the octoploid (Table 4.21). About one hundred and fifty embryos of both accessions were rescued. No germination was observed in the crossed embryos of T1 x tetraploid *P. pedicellatum.* Germination took place on the 4th day in control embryos (T1 and T2) and in 5-7 days in the crossed embryos of T1 x *P. pedicellatum* Agros 4. Just like the previous cross of T1 x *P. polystachyon,* some embryos showed early germination while some of them germinated very late even after one week. The early germinated embryos were thought to be the self-pollinated one and the embryos that showed late germination were expected to be the crossed ones and were vigorous than both the parents.

After 3-5 weeks the expected plantlets (3-4 cm) of all the crossed embryos, which showed slow growth, were transferred to root inducing media (RL) for root initiation. After 3 to 5 weeks the plantlets were transferred to L2 medium (Philips and Collins 1979) containing NAA (0.008 mg/l) and BAP (0.15 mg/l) where they developed to a 3-5 cm plantlets in 2-3 weeks with 4-6 leaves. All these crossed plantlets died after four to six months and could not be transferred to the field.

Advancement of generations:

4.3.2. Intragenomic and intraploidal interspecific hybridization (Pearl millet x *P. violaceum* and pearl millet x *P. mollisimum*):

Two primary gene pool species *P. violaceum* and *P. mollisimum* having same ploidy as diploid pearl millet were used in the interspecific hybridization. These two wild species were weedy type, had fodder value, and *P. violaceum* being source of cytoplasmic male sterility that can be transferred to the pearl millet for its improvement.

4.3.2.1. Pearl millet x *P. violaceum*: The male sterile line (81A1) of pearl millet was crossed with *P. violaceum* (PV 2433), both plants had 2n=14 chromosome number. Both the species, being compatible, produced numerous seeds. The seeds were grown in the field in the month of August 2004.

Morphological observations: Three F_1, three F_2 and three BC_1 plants were randomely selected and the data was analyzed. The plants were morphologically intermediate to both the parents for metric traits (Table 4.22, 4.23 and 4.24).

1. Number of tillers: The range for this character in F_1 was 4-10. Mean and standard deviation was recorded as 7.00 and 3.00, respectively. The range in F_2 and the BC_1 was quite lower than the F_1 and was recorded as 1-3 in F_2 and 1-7 in BC_1 plants. In F_2, the mean was 2.00 while standard deviation was 1.00. Similarly in BC_1 the mean was recorded as 4.00 and the standard deviation was 3.00.

2. Height of main tiller (cm): F_1 was found to be vigorous than the parents. This character ranged highest in BC_1 (90 -140) while range was observed to be 110 -145 in F_1 and 70 cm-100 cm in F_2. The values of mean and standard deviation were also highest in the BC_1 plants (110.0 and 26.46 respectively). The mean in F_1 was 131.67 cm and standard deviation was 18.93. In F_2, the mean was 86.67 cm and standard deviation was 15.28.

3. Number of leaves/tiller: The range was observed to be quite similar in all the three populations viz. 6-8 in F_1 and BC_1 and 6-7 in the F_2. The mean values were recorded as 7.00, 6.33, and 7.33 in F_1, F_2 and the BC_1 plants, respectively. The standard deviation was observed as 1.00 in F_1, 0.58 in F_2 and 1.15 in BC_1.

4. Flag leaf length (cm): The mean values in F_1, F_2 and BC_1 were intermediate to both the parents (26 cm in pearl millet and 11.7 cm in *P. violaceum*).. The range of flag leaf length was highest in the F_2 (10 cm-20 cm) and lowest in the F_1 (15 cm- 20 cm), BC_1 had range between 18 cm-25 cm. The mean value was highest in the BC_1 (21.00 cm), F_1 had 16.67 cm and F_2 had 15.00 cm. Standard deviation was found highest in the F_2 (5.00) while in BC_1 and F_1 the standard deviation was observed to be 3.61 cm and 2.89 cm.

5. Flag leaf width (cm): Like the flag leaf length, the mean values of flag leaf width in F_1, F_2 and BC_1 were intermediate to both the parents (2.5 cm in pearl millet and 0.4 cm in *P. violaceum*). The range varied between 2 cm- 2.5 cm in the BC_1 and 0.8 cm- 1.0 cm in the F_1. No variation was observed in the range of F_2 (1.00 cm). The mean values were recorded as 0.93 cm, 1.00 cm and 2.00 cm in F_1, F_2 and BC_1 respectively. Standard deviation was highest in the BC_1 (0.5) and in F_1 it was observed as 0.12 while F_2 had zero SD.

6. 3rd leaf length (cm): F_1 and BC_1 were vigorous than the parents for the character. The range of 3rd leaf length in F_1 was 40 cm- 50 cm, in F_2 was 25-40 and in BC_1 it was observed as 40 cm- 55 cm. Mean was highest in BC_1 (50.00 cm) and lowest in the F_2

(33.33 cm) while in F_2 it was intermediate (45.50 cm). Standard deviation was also highest in the BC_1 (8.66), while F_1 and F_2 had 5.00 and 7.64 SD.

7. 3rd leaf width (cm): High variation in range of BC_1 (1.5 cm- 2.8 cm) was recorded. In F_1 it was between 1.5 cm and 2.0 cm while in F_2, the range was recorded between 1.4 cm and 1.8 cm. The mean values in F_1, F_2, and BC_1 were recorded as 1.67 cm, 1.57 cm and 2.30 cm, respectively which were intermediate to both the parents (3 cm in pearl millet and 0.9 cm in *P. violaceum*). Highest standard deviation was observed in BC_1 (0.70) while in F_1, it was 0.29 and in F_2, it was recorded as 0.21.

8. Stem girth (cm): When compared to the parents, intermediate values of range of stem girth were observed in F_1, F_2 and the BC_1 plants. F_1 ranged between 0.4 cm and 0.6 cm and BC_1 ranged between 0.6 cm and 0.8 cm while no variation was observed in F_2 (0.3 cm). The mean was recorded as 0.50 cm, 0.30 cm and 0.70 cm in F_1, F_2 and The BC_1 plants, respectively. Standard deviation was 0.10 in F_1 and BC_1 while zero SD was observed in F_2.

9. Number of nodes/tiller: Not much variation in the range of F_1, F_2 and BC_1 plants was recorded and the range was between 6 and 8. The mean values in F_1 (7.00) and BC_1 (7.33) were higher than both the parents (5 in pearl millet and 6.7 in *P. violaceum*). F_2 had little lower mean value (6.33) than the male parent. Standard deviation in the three populations viz F_1, F_2 and BC_1 was recorded as 1.00, 0.58, and 1.15, respectively.

10. Internode length (cm): In F_1, this character ranged between 14 cm -15 cm, in F_2 between 10 cm and 15 cm and in BC_1 between 11 cm and 14 cm. F_1 was vigorous than both the parents (13 and 13.67 in female and male parent) and had mean value 14.33. F_2 had 13.00 mean value while BC_1 had 12.67. The standard deviation was recorded as 0.58 in F_1, 2.65 in F_2 and 1.53 in BC_1.

11. Peduncle length (cm): The mean values in F_1 (24.33), F_2 (20.67) and BC_1 (26.00) were recorded higher than the male parent (11.67) and lower than the female parent (30). Standard deviation in F_1 was 1.15, in F_2 was 5.51 and in BC_1 was 5.29. The range was found between 23 cm- 25 cm in F_1, 17 cm and 27 cm in F_2 and 20 cm and 30 cm in BC_1 plants.

12. Spike length (cm): The mean values of spike length were intermediate to the parents in the F_1, F_2 and BC_1 plants. The female parent had 26 cm spike length, male parent *P. violaceum* had 6.67 cm while F_1 had 13.33 cm, F_2 had 8.00 cm and BC_1 had 17.33 cm

mean value. Standard deviation was recorded as 1.15, 1.00 and 5.51 in F_1, F_2 and BC_1, respectively.

13. Spike width (cm): Just like the spike length, the spike width also had intermediate mean values in the F_1, F_2 and BC_1 than both the parents. The F_1 and BC_1 both had 1.43 cm mean and F_2 had 1.67 cm while the female parent had 3 cm and male parent had 1.13 cm spike width. Standard deviation was same in F_1 and BC_1 (0.12) and was 0.29 in F_2. The range of the character in F_1 was 1.3 cm to 1.5 cm, in F_2 was 1.5 cm to 2.0 cm and in BC_1 it was recorded as 1.3 cm to 1.5 cm.

The qualitative characters of the F_1 were predominant of the male parent (*P. violaceum*). The nodes were hairy, leaf base colour, and the awns colour was violet like the male parent instead of non hairy node, light violet leaf base colour, and yellow awns colour of the female parent (Table 4.24).

Cytological observations: On flowering, about five randomly selected plants were cytologically analyzed. Three plants showed normal meiosis and contained 2n=14 chromosome number and the average chromosomal association at diakinesis was 7_{II}. One plant showed $0.1_I + 6.95_{II}$ average chromosomal association while one another plant (20/9) showed highly abnormal meiosis. This plant showed variable number of bivalents at diakinesis like 5, 6 and 8 bivalents in about 16% of pollen mother cells. Micronucleoli and unequal distribution of chromosomes at anaphase I was observed. The pollen mitosis of this plant was also observed to be disturbed and variable chromosome numbers in the pollen were observed. Pollen stainability in all these F_1 hybrids was quite high and ranged between 85 to 95% and the range of pollen size was between 21 μm to 60 μm (Table 4.25). These F_1 hybrids were further crossed with male sterile pearl millet (81A1) to produce BC_1. The BC_1 plants segregated for morphological characters. Few randomly selected plants were cytologically analyzed and the average chromosomal association at diakinesis was found to be 7_{II} and pollen stainability was also high (above 60%) in all plants except for one which showed $0.08_I + 6.96_{II}$ chromosomal association and pollen stainability in this plant was 20%.

The F_2 plants produced were morphologically more likely towards the *P. violaceum*. On doing cytologically analysis, the average chromosomal association was observed to be 7_{II} and pollen stainability was 60%.

4.3.2.2. Pearl millet x *P. mollisimum*: The male sterile line (81A1) of pearl millet were crossed with *P. mollisimum* (IP 21782), both plants having 2n=14 chromosome number.

Both the species were also compatible and produced numerous seeds. The seeds were grown in the field in the month of August 2004.

Morphological observations: Three randomly selected F_1 plants were analyzed. Morphologically the plants were intermediate to both the parents for metric traits (Table 4.22, 4.23, 4.24).

1. Number of tillers: The range for this character in F_1 was 4-8. Mean and standard deviation was recorded as 6.00 and 2.00, respectively. The mean value was lower than either of the parents (8 in pearl millet and 8.7 in *P. mollisimum*).

2. Height of main tiller (cm): F_1 was found to be intermediate to the parents. This character ranged between 200 cm and 310 cm. The values of mean and standard deviation were recorded as 240 cm and 60 cm, respectively.

3. Number of leaves/tiller: The range was observed to be between 10 and 11. The mean value was recorded as 10.33 in F_1 which was lower than the parents (12 in pearl millet and 13.33 in *P. mollisimum*). The standard deviation was observed as 0.58 in F_1.

4. Flag leaf length (cm): The mean value in F_1 was lower (14.33 cm) than either of the parents (26 cm in pearl millet and 24 cm in *P. mollisimum*). The range of flag leaf length was recorded as 12.5 cm- 17 cm in the F_1. Standard deviation was found to be 2.36.

5. Flag leaf width (cm): Like the flag leaf length, the mean value of flag leaf width in F_1 was 1.27 cm while in parents it was recorded as 2.5 cm in pearl millet and 1.0 cm in *P. mollisimum*. The range varied between 1 cm and 1.5 cm in the F_1. Standard deviation was observed as 0.25.

6. 3rd leaf length (cm): F_1 had lower mean value (26.33 cm) of this character than the parents (40 cm in pearl millet and 45 cm in *P. mollisimum*). The range of 3rd leaf length in F_1 was 23 cm- 30 cm. Standard deviation in F_1 was 3.51.

7. 3rd leaf width (cm): In F_1 the range was observed between 1.7 cm and 2.5 cm. The mean value in $F_1$was recorded as 2.07 cm which was the intermediate value than both the parents (3 cm in pearl millet and 1.63 cm in *P. mollisimum.* Standard deviation in F_1 was observed as 0.40.

8. Stem girth (cm): When compared to the parents, intermediate values of mean of stem girth were observed in F_1. The mean in F_1 was recorded as 0.63 cm while in female parent, it was 1 cm and in male parent, it was 0.72 cm. F_1 ranged between 0.6 cm and 0.69 cm. Standard deviation was 0.05 in F_1.

9. Number of nodes/tiller: The mean values in F_1 was 10.3 while in the female parent (pearl millet) it was 5 and 11.7 in the male parent (*P. mollisimum*). Standard deviation in F_1 was recorded as 1.53. The range of the character was found to be between 9 and 12.

10. Internode length (cm): In F_1, this character ranged between 15 cm -28 cm. F_1 had quite similar mean value (21.00 cm) than the male parent (21.83 cm) and much higher than the female parent (13 cm). The standard deviation was recorded as 6.56 in F_1.

11. Peduncle length (cm): The mean values in F_1 were recorded intermediate (26.7 cm) to the parents (30 cm in female and 18.67 cm in male). Standard deviation in F_1 was 4.73. The range was found between 23 cm- 32 cm in F_1.

12. Spike length (cm): The mean values of spike length in the F_1 were intermediate to the parents. The female parent had 26 cm spike length, male parent *P. mollisimum* had 14.7 cm while F_1 had 16 cm. Standard deviation was recorded as 14.36 in F_1. The range of the character in F_1 was 11 to 19.

13. Spike width (cm): Unlike the spike length, the spike width had lower mean values in the F_1 (1.7 cm) than both the parents (3 cm in pearl millet and 1.77 cm in *P. mollisimum*). Standard deviation was 0.29 in F_1. The range of the character in F_1 was 1.5 cm to 2.0 cm.

The qualitative characters of the F_1 (just like the previous cross with *P. violaceum*) were predominant of the male parent (*P. mollisimum*), the nodes were hairy, leaf base colour, and the awns colour was dark violet like the male parent instead of non hairy node, light violet leaf base colour, and yellow awns colour in the female parent (Table 4.24). On flowering, thirteen randomly selected plants were cytologically analyzed. All showed 7_{II} chromosomal association at diakinesis except one plant that showed $0.32_I + 6.84_{II}$ at diakinesis and pollen fertility was 73.33%. Pollen size difference was noticed in all hybrids ranging from 24 µm to 51 µm and pollen stainability varied highly in all plants (40 % to 75%).

4.3.3. Intergenomic and interploidal crosses

4.3.3.1. Pearl millet x *P. squamulatum*: F_1 hybrids have previously been produced at IGFRI Jhansi involving tetraploid pearl millet and *P. squamulatum*. Two accessions of tetraploid pearl millet (viz. IG 99-748 or T1 and IG 2000-01 or T2) and three accessions of *P. squamulatum* (viz. IG 98-360 or MA, IG 98-361 or MB and IG 2000-36 or MC) were utilized, that included two F_1 hybrids (named H1 and H2) from *P. glaucum* T1 x

P. squamulatum MA cross. H1 has previously been characterized to reproduce via sexual mode, while H2 was obligate apomictic in mode of reproduction. Three more F_1 hybrids were produced named H3, H4 and H5. H3 was utilized by crossing T1 with MC while H4 and H5 were utilized by crossing T2 and MC. H1 and H2 were crossed in all possible directions i.e. H1 x T1, T1 x H1, H1 x T2, T2 x H1, T1 x H2 and T2 x H2 to produce BC_1 generations. 24 BC_1 hybrids between H1 x T1, 4 hybrids between H1 x T2, 40 hybrids between T1 x H1, 7 hybrids between T1 x H2, 16 hybrids between T2 x H1 and 32 hybrids between T2 x H2 were grown. All of these BC_1 hybrids contained 2n=35 chromosomes, 21 from pearl millet and 14 from *P. squamulatum*. F_1 and BC_1 generations were grown and studied on the basis of morphological, cytological and biochemical attributes and further utilized in the hybridization to produce F_2, BC_1 and Sib populations.

4.3.3.1.1. Morphological characterization : The parents viz. tetraploid pearl millet (T1) and *P. squamulatum* MA, F_1 hybrids and the BC_1 plants were evaluated for various morphological traits. To produce BC_1 generation, two accessions of pearl millet (T1 and T2) were utilized in the crossing with F_1 hybrids. 61 BC_1 plants were analyzed morphologically (Table 4.26). The mean, coefficient of variation, genotypic coefficient of variation, phenotypic coefficient of variation, genetic advance values and heritability were observed for various traits. The results showed a considerable extent of variation for all the traits studied (Plate 8).

Variability for quantitative traits (Table 4.27, Plate 8): The various metric traits were subjected to statistical analysis to ascertain the level of genetic variability.

Cluster analysis: Non- Hierarchical Euclidian cluster analysis technique was used to determine the extent of variation/similarity among sixty six plants including parents, F_1 hybrids and BC_1 generation based on thirteen morphological traits *viz.*, number of tillers, height of main tiller, peduncle length, spike length, spike width, number of leaves, flag leaf length, flag leaf width, 3rd leaf length, 3rd leaf width, stem girth, number of nodes and internode length. The analysis resulted in identification of eight clusters (Table 4.27). Cluster 7 was the largest one with total nineteen plants, followed by cluster no. 5 with eleven plants, cluster 1 and cluster 8 with 10 plants in each, cluster 2 with seven plants and cluster 3 with six plants. Cluster 4 had only two plants (T1 and T2) while cluster six consists of a single plant (*P. squamulatum* IG 98-360).

The maximum distance between cluster centroids was observed between cluster 4 and 6 (13.081) followed by cluster 3 and 6 (11.672), cluster 3 and 4 (10.325). However, minimum distance was observed between cluster 5 and 7 (1.898). the average distance between the cluster members from cluster centroids indicated that plants in cluster 2 showed wide diversity (2.4) followed by cluster 8 (2.2) and cluster 3 (2.1). The members of cluster 7 were found to be closely related (1.6) (Table 4.28).

The different characters studied are described below (Table 4.29 and 4.30):

1. Number of tillers: The range of number of tillers was lower in the F_1 hybrids (3.0 and 15.3 in H1 and H2 respectively) than the male parent (106.67 in *P. squamulatum*). The value observed in the female parent (T1) was 4.46. All BC_1 plants ranged between 1.5 (MP 5/41 T1H1) and 28.33 (MP 2/29 H1T1). The coefficient of variation was the highest for this character (37.5). Among the BC_1 groups, H1T1 ranged between 4.33 and 28.33, mean was found to be 10.12 and standard deviation for the character was 5.64. In H1T2, the number of tillers ranged between 9.50 and 18.33. The mean and standard deviation was observed as 13.92 and 6.25 respectively. The plants of the cross T1H1 ranged between 1.50 and 28.0 and the mean was 12.38. Standard deviation was the highest of all viz. the parents, hybrids and the other groups of BC_1 and was recorded as 8.32. T2H1 plants were observed to have range between 9.50 and 22.67. The mean and standard deviation was 15.87 and 4.47 respectively. The groups of BC_1 involving H2 as male parent showed range between 7.67 and 25.0 (T2H2) and 12.50 to 24.61 (T1H2). The mean and standard deviation in T2H2 was 15.09 and 4.84 while in T1H2 it was 16.38 and 5.72 respectively.

2. Height of main tiller (cm): The height of the main tiller in T1 was recorded as 136.67 cm and in *P. squamulatum* as 186.33 cm. Both the F_1 hybrids had lower values than the parents (80.0 cm in H1 and 104.0 cm in H2). The coefficient of variation was 15.9 for this character. The range of the character was highest in the BC_1 H1T1 and was observed between 44.62 cm and 171.00 cm and lowest in H1T2 between 119.00 cm and 188.00 cm. The lowest mean of the character among all the BC_1 groups was 129.85 in T1H1 while the highest mean was observed in T2H2 211.91. Standard deviation was found lowest in T1H1 (27.12) and highest in H1T2 (48.79).

3. Peduncle length (cm): The peduncle length was observed to be 28.00 cm in T1 and 57.00 cm in *P. squamulatum.* Both the F_1 hybrids showed intermediate values than the

parents with 30.7 cm in H1 and 30.3 cm in H2. The longest peduncle (56.33 cm) was observed in the BC_1 MP 5/35 (T1H1) and the shortest (24.0 cm) was observed in MP 5/29 (T1H1). The coefficient of variation being the least among all (11.6). The lowest range of peduncle length was observed in T1H2 between 37.61- 42.17 and the highest was in T1H1 between 24.0- 56.33. Lowest mean was observed as 35.13 (T1H1) and lowest standard deviation was 2.05 (T1H2). The highest mean and standard deviation was observed as 42.67 and 16.50 respectively, both in H1T2.

4. Spike length (cm): In the parents spike length was observed as 22.33 cm in T1 and 31.67 cm *P. squamulatum*. H1 showed shorter spike than both the parents while in H2 longer spike than the parents was observed (26.3 cm).BC_1 plants showed very high variability for the character with the shortest spike (14.3 cm) in MP 2/12 (T1H1) and the longest spike (34.0 cm) in MP 2/23 (T2H2). The coefficient of variation was found to be 13.7. Among the groups, Highest variability for range of spike length was recorded in H1T1 (10.81 cm- 27.00 cm) while the lowest range was observed in T1H2 (24.67 cm- 33.33 cm). T2H2 plants represented the highest mean (29.30) and the lowest standard deviation (2.77). Lowest mean for the character was observed in the plants of T1H1 (20.50). Highest standard deviation was found in H1T2 (7.31).

5. Spike width (cm): *P. squamulatum* was vigorous with 2.00 cm spike width than the female parent (tetraploid pearl millet T1) with 1.13 cm while T2 had 3.00 cm spike width. Both the F_1 hybrids had spike width 1.6 cm (H1) and 1.9 cm (H2). The spike width in BC_1 progeny ranged between 1.20 cm (MP 2/16 T1H1) and 2.8 cm (MP 4/33 (T2H2). The coefficient of variation was observed to be 14.8. In the various groups of BC_1 plants, the spike width ranged lowest in H1T2 (1.55 cm- 1.87 cm) and the character ranged highest in T1H1 (0.18 cm- 1.97 cm). The lowest mean and standard deviation was recorded as 1.55 (T1H1) and 0.18 (T1H1) respectively. Highest mean for the character was 2.19 (T2H2) and highest standard deviation was 0.33 (T2H1).

6. Number of leaves: The range of number of leaves in the parents was from 6.33 (T1) to 10.67 (*P. squamulatum*). The H1 had lower number of leaves (6.0) than the parents while H2 showed intermediate value (8.7). In the BC_1 progeny the variation was between 4.0 (MP 5/41 T1H1) and 11.3 (MP 2/27 T2H2). The coefficient of variation for this character was observed to be 12.7. Not much variation was observed for the range of the character in the BC_1 groups except H1T1 in which number of leaves ranged between

3.66 and 8.00. The lowest mean (6.25) was in T1H1 and lowest standard deviation (0.94) was in H1T2. Highest mean (8.58) and standard deviation (1.75) was recorded in T2H2.

7. Flag leaf length (cm): Unlike other characters, this character had low value (31.33 cm) in the male parent *P. squamulatum* than the female (42.7 cm). Both F_1 hybrids had lower values than the parents (16.7 cm in H1 and 22.8 cm in H2). The variation in BC_1 generation was very high and was recorded between 7.0 cm (MP 5/41 (T1H1) and 45.33 cm in (MP 4/33 T2H2). The coefficient of variation was 21.8. Among the BC_1 groups, the range of flag leaf length varied lowest in H1T2 (20.75- 27.61) and highest in T2H2 (16.27-45.32). Lowest mean was 20.35 in T1H1 while highest was 27.60 in T2H2. The value of standard deviation was lowest (4.89) in H1T2 and highest (7.57) in T1H2.

8. Flag leaf width (cm): Both the F_1 hybrids had lower values of the flag leaf width than the parents (0.9 cm and 1.1 cm in H1 and H2 while 3.5 cm and 1.40 cm in T1 and *P. squamulatum* respectively). Flag leaf width ranged between 0.7 cm (MP 5/41 T1H1) and 2.3 cm (MP 5/15 H1T1 and MP 4/30 T2H1). The coefficient of variation was found to be 1.57. Among the groups of all BC_1 plants the lowest and highest range for the character was 1.33 cm- 1.40 cm (H1T2) and 0.46 cm- 2.30 cm (H1T1) respectively. The lowest and highest mean values were 1.11 (T1H2) and 1.72 (T2H1) and that of standard deviation were 0.05 (H1T2) and 0.45 (H1T1) respectively.

9. 3rd leaf length (cm): The female parent (T1) was vigorous (71.70 cm) than the male parent *P. squamulatum* (68.67 cm) for 3rd leaf length. Both the hybrids had values lower than the parents (43.2 cm and 44.3 cm in H1 and H2 respectively). BC_1 MP 5/41 (T1H1) had the shortest 3rd leaf (19.3 cm) and MP 2/23 (T2H2) had the longest (70.8 cm). The coefficient of variation was 19.3. On comparing the groups of BC_1 plants, it was found that the range varied highest in T1H1 between 19.25 cm and 60.17 cm and lowest variation was observed in H1T2 between 41.25 cm and 57.50 cm. Mean values of 3rd leaf length varied from 39.58 cm (T1H2) to 52.15 cm (T2H2) and standard deviation of the character varied from 7.89 (T1H2) to 11.49 (H1T2).

10. 3rd leaf width (cm): Both F_1 hybrids had lower values of 3rd leaf width (1.0 cm in H1 and 1.2 cm in H2) than both the parents (3.67 cm in T1 and 1.6 cm in *P. squamulatum*). BC_1 ranged between 0.90 (MP 5/41 T1H1) and 3. cm (MP 4/30 T2H1). The coefficient of variation was found to be 18.0. The 3rd leaf width among the various groups ranged lowest 1.50 cm- 1.93 cm in H1T2 and highest 0.75 cm- 3.0 cm in

T2H1. The mean value ranged between 1.24 cm (T1H2) and 1.97 (T2H2) and the standard deviation ranged between 0.24 (T1H2) and 0.62 (T2H1).

11. Stem girth (cm): T1 had 1.67 cm stem girth and *P. squamulatum* had 0.57 cm stem girth. H1 showed lower value (0.5 cm) than both the parents while intermediate value (0.6 cm) was observed in H2. All BC_1 contained lower values for the trait than the female parent. The range of stem girth in BC_1 was lowest (0.5 cm) in MP 2/31 (T1H1) and highest (0.9 cm) in MP 4/33 (T2H2). The coefficient of variation was 0.7. The range of stem girth in the various groups of BC_1 plants was found to be lowest 0.60 cm- 0.70 cm in H1T2 and highest 0.29 cm- 0.83 cm in H1T1. The lowest mean value for stem girth was observed in T1H1 (0.59) and the highest mean value for the character was observed in T2H2 (0.78). Similarly, lowest standard deviation was observed in H1T2 (0.07) and the highest in two groups viz.H1T1 and T2H2 (0.12).

12. Number of nodes: The number of nodes showed lower value (5.3) in H1 than the parents (6.0 in T1 and 10.67 in *P. squamulatum*) while the H2 showed intermediate value (7.0 The lowest number of nodes (4.3) in the BC_1 plant was observed in MP 5/20 (H1T1) while the highest (16.8) was observed in the BC_1 plant MP 4/3 (T2H1). The coefficient of variation for this character was observed to be 15.5. The range of number of nodes in the BC_1 groups was lowest in H1T2 (6.60- 7.0) and highest in T2H1 (6.0-16.83). The mean values varied from 5.52 in H1T1 to 8.13 in T2H1 and the standard deviation was found to be between 0.35 (H1T2) and 3.14 (T2H1).

13. Internode length (cm): *P. squamulatum* had longer internodes (16.0 cm) than T1 (15.33 cm). Lower values than the parents were observed in the both the F_1 hybrids (11.7 cm in H1 and 7.8 cm in H2). The variation in BC_1 was observed between 4.00 cm (MP 5/41 T1H1) and 23.7cm (MP 5/35 T1H1). The coefficient of variation for this character was observed 15.2. among the various groups of BC_1 populations, the internode length was observed to have range between 14.33 cm- 16.33 cm (T1H2) and 4.0 cm- 23.67 (T1H1). The mean value of the character varied from 14.82 cm (T1H1) to 20.04 cm (H1T2) and the standard deviation of the character showed high variation viz. from 0.92 (T1H2) to 5.06 (T1H1)

Mean performance and variability:

The range, overall mean, and coefficient of variation at phenotypic and genotypic levels for all the morphological traits of parents F_1 hybrids and the BC_1 is presented in Table 4.31.

The wide range of variation was observed for all the characters like number of tillers (1.33- 106.67), height of main tiller (73.50- 270.0 cm), peduncle length (24.0 -56.33 cm), spike length (14.33- 34.0 cm), spike width (1.0- 2.80 cm), number of leaves (4.0-11.33), flag leaf length (7.0- 45.33 cm), flag leaf width (0.70- 3.50 cm), 3rd leaf length (19.25- 71.67 cm), 3rd leaf width (0.90- 4.0 cm), stem girth (0.47- 1.67 cm), number of nodes (4.33- 16.83) and internode length (4.0 -29.0 cm). The genotypic coefficient of variation was lower than the corresponding phenotypic coefficient of variation for all the characters studied. The highest phenotypic coefficient of variation was observed in the case of number of tillers per plant (98.70). Relatively high phenotypic coefficient of variation was registered for several characters such as flag leaf width (34.88), 3rd leaf width (34.49), flag leaf length (33.62), stem girth (30.87), height of main tiller (30.56), number of nodes (29.56), 3rd leaf length (28.05), internode length (27.82), spike width (23.16), number of leaves (22.75), spike length (22.17) and the peduncle length had the lowest phenotypic coefficient of variation (20.75).

Similarly the genotypic coefficient of variation was also highest for the number of tillers per plant (91.31) followed by 3rd leaf width (29.40), flag leaf width (27.85), height of main tiller (26.05), stem girth (25.70), flag leaf length (25.58), number of nodes (25.17), Internode length (23.32), 3rd leaf length (20.40), number of leaves (18.87), spike width (17.83), spike length (17.40) and peduncle length had the least genotypic variation among all (17.18).

Heritability and Genetic Advance:

The heritability can be defined as the ratio of genotypic variance to total or phenotypic variance (broad sense) and the ratio of additive genetic variance to phenotypic variance (narrow sense). The heritability estimates in broad sense were obtained from analysis of variance from 13 metric traits and the results are given in the Table 4.31. The highest heritability was recorded for number of tillers (0.86) while moderate heritability was recorded for height of main tiller (0.73), 3rd leaf width (0.73), number of nodes (0.73), internode length (0.70), stem girth (0.69), number of leaves (0.69), peduncle length (0.69), flag leaf width (0.64), spike length (0.62), spike width (0.59), flag leaf length (0.58) and the lowest heritability was recorded for 3rd leaf length (0.53).

Genetic advance is the improvement in mean genotypic value of selected plants over the parental population. The highest value of genetic advance was recorded

for height of main tiller (71.45) and the lowest value of genetic advance was observed for stem girth (0.30). The moderate values were recorded for number of tillers (24.63) followed by 3rd leaf length (13.73), peduncle length (11.03), flag leaf length (9.68), spike length (6.77), internode length (6.31), number of nodes (2.98), number of leaves (2.34), 3rd leaf width (0.93), flag leaf width (0.72), spike width (0.53) and stem girth (0.30).

Correlation studies: The correlation studies were carried out among the 13 metric traits of the parents, F_1 hybrids and the BC_1 generation.

Genotypic correlation (Table 4.32): At the genotypic level, positive correlation was observed for the number of tillers with height of main tiller (r=0.460), number of leaves and internode length. Negative correlation of number of tillers was observed with spike width (r=-0.464). Very high positive correlation was observed for height of main tiller with the number of leaves (r = 0.818) followed by peduncle length (r=0.615), spike length, internode length and 3rd leaf length. Positive correlation of height of main tiller was observed with flag leaf length and number of nodes. Flag leaf length had a very high positive correlation with 3rd leaf length (r=0.848) followed by flag leaf width, stem girth, 3rd leaf width, and internode length. The highest positive correlation observed among all was of flag leaf width with 3rd leaf width and stem girth (r = 0.964) and a high correlation with 3rd leaf length. However, significant correlation was obtained with internode length (r = 0.360). High positive correlation of 3rd leaf length was obtained with 3rd leaf width (r=0.7), stem girth and internode length. 3rd leaf width also had a very high positive correlation with stem girth. However, number of nodes had a negative correlation with internode length (r= -0.276).

Phenotypic correlation (Table 4.33): At the phenotypic level, number of tillers had a significant positive correlation with height of main tiller (r = 0.391), number of leaves and internode length while number of tillers had a negative correlation with spike width (r= - 0.344). A very high positive correlation of height of main tiller was observed with number of leaves (r=0.713), peduncle length (0.580) and spike length. Positive correlation of height of main tiller was with internode length (r = 0.480), number of nodes, 3rd leaf length, flag leaf length while significant correlation was with 3rd leaf width, stem girth and flag leaf width. Peduncle length showed high positive correlation with spike length and internode length and positive correlation with 3rd leaf length and significant correlation with flag leaf length. Spike length had a high positive correlation

with spike width and positive correlation with 3rd leaf length, flag leaf length, and significant correlation with flag leaf width, 3rd leaf width and number of leaves. A high positive correlation of spike width was obtained with stem girth and flag leaf width while positive correlation was observed with 3rd leaf width, flag leaf length and significant correlation with 3rd leaf length. Number of leaves had a high positive correlation with number of nodes and significant correlation with stem girth. Flag leaf length had a very high correlation with 3rd leaf length, flag leaf width and 3rd leaf width. However, positive and significant correlation was noted with stem girth and internode length respectively. The highest value of very high positive correlation was observed for flag leaf width with 3rd leaf width (r = 0.815) followed by stem girth and high positive correlation with 3rd leaf length. Again, high positive correlation of 3rd leaf length was observed with 3rd leaf width. A positive correlation was observed with stem girth and a significant correlation with internode length. 3rd leaf width had a high positive correlation with stem girth and significant correlation with internode length.

Qualitative traits: The following qualitative traits were studied in the parents, F_1 hybrids and BC_1 progeny of pearl millet and *P. squamulatum* (Table 4.34, Plate 8).

Node hairiness: The female parents T1 and T2 had hairy node while the node of male parent *P. squamulatum* IG 98-360 was non-hairy. The character segregated in F_1 hybrids and the BC_1 generations. Two F_1 hybrids H1 and H2 had hairy node while the rest of the three had less hairy node. In BC_1 generations 95% of the H1T1 plants had hairy node and the rest 5% had less hairy node. In T1H1 85% plants had hairy while 15% had less hairy nodes. The rest of the BC_1 generations i.e. H1T2, T2H1, T1H2 and T2H2 showed 100% hairy nodes. No F_1 and BC_1 plant was observed to have non-hairy nodes.

Coloured ring at node: Both the tetraploid accessions of pearl millet differ for this character as T1 had yellow node while the T2 had light violet node colour. In F_1 hybrids the character segregated and H1 had yellow nodes as both of its parents while H2 had violet colour. The other three F_1 hybrids had light violet nodes. In H1T1, no yellow nodes were observed and percentage of the violet and light violet colour was found to be 70% and 30% respectively. T1H1 and T2H1 had high percentage of yellow nodes i.e. 85% and 72.7% and relatively low percentage light violet nodes i.e. 15% and 27.3% respectively. All H1T2 plants had yellow nodes. The dark violet colour was preponderant in T1H2 (75%) while the light violet colour was observed in only 25%

plants. Similarly T2H2 showed the presence of 30% each of violet and dark violet nodes and 40% of light violet nodes.

Leaf base colour: Yellow leaf base colour was found to be predominant in both accessions of pearl millet, *P. squamulatum* and H1. H2 and H4 had yellow + violet leaf base, H3 had violet and H5 had light violet leaf base. Both the category of BC_1 viz. H1T1 and T1H1 plants had yellow leaf base in 60% and 70%, yellow +violet in 30% and 25%, and violet in 10% and 5% plants respectively. 33.3% yellow and 66.6% yellow +violet character was observed in H1T2 BC_1 plants. In T2H1, 45.5% yellow, 27.3% yellow +violet, 18.18 violet and 9.1% light violet leaf base colour was observed. T1H2 posses 25% yellow +violet and 75% violet leaf base and T2H2 posseses both yellow +violet and violet leaf base in 30% and 70%.

Leaf hairiness: The parents (pearl millet and *P. squamulatum*) and F_1 hybrids had hairy leaf except H3 which had less hairy leaves. This character segregated in H1T1 with 50-50% for leaf hairiness and less hairiness. In T1H1 it was 35% and 65%, in H1T2 it was 33.3% and 66.6%, in T2H1 45.45% and 54.5% and all plants of T1H2 and T2H2 had hairy leaves.

Leaf orientation: Parents (pearl millet and *P. squamulatum*) and two hybrids H2 and H3 had semi drooping leaf orientation, H1 and H4 had straight and H5 had drooping leaves. H1T1 segregated into 10% straight, 20% semi drooping, and 70% drooping leaves in T1H1 it was 15% straight and 85% semi drooping. Similarly H1T2 had 33.3% and 66.6% in T2H1 18.2% and 81.8% straight and semi drooping leaf orientation. T1H2 had 100% drooping leaves while leaf orientation segregated in T2H2 with 10% straight, 50% semi drooping and 40% drooping.

Peripheral sheath colour: Yellow colour of parents was expressed in F_1 hybrids viz. H1 and H4 while in H2, H3 and H5, violet colour appeared. Yellow peripheral sheath colour was expressed in BC_1 represented by H1T1, T1H1, H1T2 and T2H1 with 75%, 90%, 100% and 81.8% respectively. The expressivity of violet colour was observed in 10%, 5% plants in H1T1 and T1H1 while it is observed in 100% plants in each T1H2 and T2H2. Yellow +violet colour appeared in 15% in H1T1 and 5% in T1H1 and 18.2% in T2H1 plants.

Awn colour: Three F_1 had yellow and one each was with light violet and dark violet awn colour. The highest frequency of yellow colour awn were observed in H1T1 with 60% while violet 30% and light violet with 10%. T1H1 showed 75% yellow awn, light

violet and dark violet in 10% plants each and 5% awn were violet coloured. T2H1 had 18.18% light violet, 54.5% yellow, and 27.3% violet coloured awn. T1H2 had 75% violet and 25% dark violet coloured awn. Yellow coloured awn were absent in T2H2 and violet colour was predominant in 80% while light violet and dark violet were observed in 10% each.

Stigma colour: The violet colour of stigma which was the characteristic feature of male parent *P. squamulatum* was predominant in the F_1 hybrids except H1 which had white stigma. The character segregated in BC_1 involving H2 as one of its parent's i.e.T1H2 and T2H2 in 1:1 ratio. All other BC_1 had only white stigma.

Stigma (bifid / trifid): The trifid stigma was found only in the male parent and was absent in the female parent. Only H4 had bifid +trifid stigma and rest of the F_1 hybrids had bifid stigma. In BC_1 generation only 5% of T1H1 plants had bifid +trifid stigma and the rest had bifid stigma. No other BC_1 plants were observed to have trifid stigma.

4.3.3.1.2. Cytological description of interspecific hybrids (F_1, F_2, BC_1 and Sibs) between pearl millet and *P. squamulatum*.

Interspecific hybrids were produced by crossing sexual pearl millet (2n=(4x)=28) with apomictic *P. squamulatum* (2n=56). Five F_1 hybrids were studied, two hybrids from pearl millet IG 99-748 (or T1) x *P. squamulatum* IG 98-360 cross which were denoted as H1 and H2, one from T1 x *P. squamulatum* IG 2000-36 (H3) and two F_1 hybrids viz. H4 and H5 from pearl millet IG 2000-01(or T2) x *P. squamulatum* IG 2000-36). All F_1 hybrids contained 2n=42 chromosomes, 14 from pearl millet and 28 from

P. squamulatum. Cytology of the parents T1, T2, *P. squamulatum* IG 98-360 and IG 2000-36 was discussed earlier in the species section.

BC_1 generation was produced by crossing T1 and T2 with F_1 hybrids H1 and H2 in all possible directions . All of these BC_1 hybrids contained 2n=35 chromosomes, 21 from pearl millet and 14 from *P. squamulatum.* All the F_1 and the BC_1 plants were analyzed and confirmed by flow-cytometry (Figure 3). Description of these crosses is given in Figure 10. The average chromosomal association at diakinesis and other cytological observations are given in Table 4.35 (Plate 9 and 10). The diagrammatical representation of $F_1$and BC_1 cross is given below:

***P. glaucum* (2*n* = 4*x* = 28):** Two lines of induced tetraploid *P. glaucum* were used in hybridization program. T1 was raised in I.G.F.R.I. in the year 1999 where as T2 was

acquired from USDA (Hanna *et al.* 1976). Meiotic analysis revealed a higher multivalent frequency in T1 as compared to T2. The meiosis is typical of induced tetraploids, showing association from univalents to quadrivalents. Average chromosomal associations were found to be $1.2_{I} + 7.16_{II} + 0.96_{III} + 2.4_{IV}$ and $0.35_{I} + 10.15_{II} + 0.25_{III} + 1.65_{IV}$ in T_1 and T2 respectively (Table 4.35). Percentage chromosomes involved in bivalent configuration was 51.4 and 72.5 in T1 and T2 respectively.

***P. squamulatum* (2*n* = 8*x* = 56):** Three accessions of *P. squamulatum* (2*n* = 8*x* = 56) were analyzed for meiotic system. The accessions showed various configurations ranging from univalents to hexavalents. However majority of chromosomes associated as bivalents. As is clear from Table 4.35. The average chromosomal associations of *P. squamulatum* accessions IG 98-360, IG 98-361 and IG 2000-36 are $22.3_{II} + 0.25_{III} + 1.75_{IV} + 0.05_{V} + 0.6_{VI}$; $2.85_{I} + 15.65_{II} + 1.7_{III} + 3.25_{IV} + 0.15_{V} + 0.5_{VI}$ and $0.3_{I} + 19.8_{II} + 1.5_{III} + 2.9_{IV}$. The percentage of chromosomes involved in bivalent configuration being 79.64, 55.89, 70.71 respectively for the three accessions.

F_1 hybrids (*P. glaucum* x *P. squamulatum*; 2*n* = 42): Cytological analysis of five F_1 hybrids revealed a chromosome number of 2n = 42 (14G + 28S) as expected. Details of meiotic system (Table 4.35, Plate 9) revealed a near normal meiosis with predominant bivalent configuration. The percentage of chromosomes involved in bivalent configurations in different pollen mother cells varied from 82.66 to 100. The hybrids were observed to be highly male and female fertile with regular seed formation and perennial growth habit. F_1 hybrids were named as H1, H2, H3, H4 and H5. All were perennial and high tillering like the male parent. By progeny analysis, it was found that out of these five hybrids, H1 reproduced sexually while the other reproduced by apomixis. Cytological details of the hybrids is as follows:

F_1 hybrids have previously been produced at IGFRI Jhansi involving tetraploid pearl millet and *P. squamulatum.* Two accessions of tetraploid pearl millet (viz. IG 99-748 or T1 and IG 2000-01 or T2) and three accessions of *P. squamulatum* (viz. IG 98-360 or MA, IG 98-361 or MB and IG 2000-36 or MC) were utilized, that included two F_1 hybrids (named H1 and H2) from *P. glaucum* T1 x *P. squamulatum* MA cross. H1 has previously been characterized to reproduce via sexual mode, while H2 was obligate apomictic in mode of reproduction. Three more F_1 hybrids were produced named H3, H4 and H5. H3 was utilized by crossing T1 with MC while H4 and H5 were utilized by crossing T2 and MC.

1. T1MA: Two F_1 hybrids have previously been produced at IGFRI Jhansi involving tetraploid pearl millet (T1) and *P. squamulatum* IG 98-360 (or MA). These F_1 hybrids were named as H1 and H2. H1 has previously been characterized to reproduce via sexual mode, while H2 was obligate apomictic in mode of reproduction. As given in the pictures 13-15 (Plate 9) this hybrid contained 2n=42 chromosomes. In H1, the average chromosomal configuration was recorded as $3.12_{I} + 17.36_{II} + 0.8_{III} + 0.44_{IV}$. Quadrivalents varied from 0-1, trivalents from 0-3 and bivalents ranged between 13-21 while univalents ranged from 0 to 8. The chiasmata frequency was observed to be 24.3 and number of chiasmata per cell ranged from 18 to 32. Equal chromosome segregation of 21:21 chromosomes was observed at anaphase I. The pollen stainability was observed to be 84%. Mean pollen size was 42.6 µm and range was 36 µm-51 µm.

The average chromosomal configuration in H2 was $0.47_{I} + 19.16_{II} + 0.42_{III} + 0.48_{IV}$. The range of univalents was 0 to 6, bivalents ranged from 12 to 21 and trivalents and quadrivalents from 0 to 4 each. The bivalents percentage was recorded as 91.24 which showed good stability of this hybrid. The pollen stainability was recorded as 64%. Mean pollen size was 31.4 µm and range was 36 µm- 48 µm.

2. T1MC: Single hybrid (2n=42) named H3 was produced by pollinating pearl millet (T1) with *P. squamulatum* (IG 2000-36 or MC). Average chromosomal configuration in this hybrid was $2.00_{I} + 19.4_{II} + 0.2_{III} + 0.15_{IV}$. The bivalent percentage was 92.38. Chiasmata frequency was 36.45. Chiasmata ranged from 29 to 42. Pollen stainability was observed as 29 %. Mean pollen size was found to be 46.5 µm and range was 39 µm -57 µm.

3. T2MC: Two hybrids were developed by crossing pearl millet (T2) and *P. squamulatum* (IG 2000-36). These were named as H4 and H5. Average chromosomal configuration in H4 was $5.32_{I} + 17.84_{II} + 0.28_{III} + 0.04_{IV}$. The bivalent percentage was 84.9, which is more than H1 but less than the other 3 hybrids (H2, H3 and H5). The average of chiasmata was 30.6 and chiasmata per pollen mother cell range from 25-34. Pollen stainability was recorded as 30%. Mean pollen size was found to be 46.2 µm and range was 39 µm-57 µm. H5 was an ideal hybrid with an average chromosomal configuration of 21_{II}. The average of chiasmata was 41.15 and chiasmata range was 34-42. Pollen stainability was recorded as 74%. Mean pollen size was found to be 39.6 µm and range was 33 µm - 45 µm.

BC_1 Progenies: Out of 125 BC_1 plants raised, cytological analysis of 42 BC_1 plants (randomly selected) was carried out. All the plants analysed had a chromosomal constitution of 2n =35 as expected (Table 4.35, Plate 10). The contribution being 21 from *P. glaucum* and 14 from *P. squamulatum*. Male and female fertility of all BC_1 plants is given in Table 4.36. Percentage of associations observed maximum times in pearl millet x *P. squamulatum* generations was presented in Table 4.37 (Fig. 4). Details of chromosome configurations are as follows:

Backcrosses involving H1

I. H1T1 (*2n*=35): 24 BC_1 plants were grown out of which 18 were cytologically analyzed (Plate 10, picture 1-11). The average chromosomal configuration in 399 pollen mother cells of 18 hybrids was recorded as $4.43_I + 12.07_{II} + 1.619_{III} + 0.323_{IV} + 0.002_V + 0.02_{VI}$. Chromosome constitution of 2n=35 consist of three sets of *P. glaucum* and two sets of 7 from *P. squamulatum* (21G+14S). The meiosis showed multivalent configurations like quadrivalents and trivalents in most of the plants. In two out of eighteen plants higher associations like hexavalents and pentavalents were also observed. Bivalent configurations however were predominant with 54.11 to 83.43 percent chromosomes associated as bivalents. The bivalent percentage in all these 18 hybrids was 68.97%. MP 5/14 had the highest bivalent frequency of 83.43% followed by MP 5/20 with 82.45%. Ten BC_1 hybrids had bivalent percentage more than 70 % and rest 8 hybrids had less than 70%. The lowest bivalent percentage was 54.11% in MP 5/7 and also hexavalents were unusually found in this plant. No other hybrid of this cross showed hexavalent formation at diakinesis. Pentavalent was observed in only in one plant. Meiotic abnormalities as expected in hybrids were commonly observed. Early and late disjunction of chromosomes at metaphase I and II (up to 14 chromosomes), laggards at anaphase I (up to 8), tripolar anaphase I, unequal chromosome separation and chromosome bridges at diad, presence of micronuclei are some of the meiotic abnormalities observed. In some cases nucleolus appeared at diad stage. The pooled average chiasma in all these 18 hybrids was 21.31 and range was recorded as 12 to 29. One hybrid MP 5/5, out of 19 hybrids, had chromosome stickiness at diakinesis. Because of this, chromosomal association at diakinesis in this plant could not be observed. Other abnormalities like abnormal spindle formation, laggards at anaphase I were also observed. Triad to hexad formation was found. At tetrad and triad, 13+14+3+5,

?+?+?+2, 8+12+15 and 5+10+20 separation of chromosomes was recorded ('?' denotes the uncountable number of chromosomes due to clumping of the chromosomes).

Surprisingly MP 5/7 had the highest pollen stainability i.e. 47.12%. Pollen size had the highest mean value 48.01 µm and range of 36 µm-54 µm. Out of 18 only one plant MP 5/8 was male sterile. The lowest pollen stainability was 5.9% in MP 5/12. The pollen stainability range was estimated from 5.9% to 47.12%. All hybrids were perennial except one plant that died in the second year.

II. T1H1 (2*n* = 35): 40 hybrid plants were grown from this cross. Six plants from this cross representing reciprocal cross of H1T1 were studied with chromosomal constitution of 2n = 35 (Plate 10, picture 12-14). Average chromosomal configurations were 4.1_{I} + 11.81_{II} + 1.688_{III} + 0.472_{IV} + 0.008_{V} + 0.02_{VI} + 0.008_{VII} + 0.02_{VIII}. Meiosis like its reciprocal, was represented mostly by multivalents such as quadrivalents and trivalents. However one plant showed the existence of multivalents like octavalents, heptavalents and hexavalents, in this plant 63.43% chromosomes were associated as bivalents. The lowest bivalent percentage was noted in MP 5/29 (56.74%) and the highest was in MP 5/41 (80.86%). Upto 10 univalents were observed at metaphase I. Average Chiasma were recorded to be 21.67 and range was from 11 to 30. The lowest average chiasma was observed in MP 5/36 with 18.55 and range of 11-26 while the highest average chiasma frequency was recorded in MP 5/40 with 23.55 and range of 19-27.Three out of six plants were identified as male sterile while the rest of three plants MP 5/29, MP 5/35 and MP 5/40 had pollen stainability of 14.22, 25.21 and 29.34 percent respectively.

III. T2H1 (2*n* = 35): Total 16 hybrid plants were grown out of which seven BC_1 plants of the combinations were worked out cytologically. The plants showed a uniform chromosomal constitution of 2n = 35 (Plate 10, picture 19-22). The average chromosome configuration in 161 pollen mother cells was recorded as 5.29_{I} + 12.27_{II} + 1.248_{III} + 0.354_{IV} + 0.01_{VI}. The percentage of chromosomes associated as bivalents was 47.77 to 82.86 % in different plants. Out of these 8, hybrids one plant viz. MP 4/10 had stickiness of chromosomes at diakinesis and metaphase I. The bivalent percentage in the 7 hybrids (except MP 4/10) was 70.09% which was more than the bivalent percentage of T1H1. The highest bivalent frequency of 82.86% was recorded in MP 4/3. The lowest bivalent percentage was 47.77% in MP 4/6 and also hexavalents were unusually found in this hybrid. No other hybrid of this cross showed hexavalent formation at diakinesis. Meiotic abnormalities as expected in hybrids were commonly observed. Staining difference

between the genomes was observed. Early and late disjunction of chromosomes at metaphase I and II was also observed. Up to 8 univalents and bivalents to quadrivalents were seen at metaphase I. Laggards were also found at anaphase I, Unequal chromosome separation (17+18, 16+17, 16+18, 15+17, 13+14, 14+17, 14+15, 15+16, 13+15, 14+19, 15+18, 16+16, 14+18) was recorded. Precocious division at anaphase I, and chromosome bridges at diad, tripolar anaphase II, presence of micronuclei were also observed. One chromosome was found lagging in about 50% pollen mother cells. Precocious mitotic division in tetrad was also observed. The average chiasma in 7 hybrids was 20.70 and range was recorded from 9 to 28.

One hybrid viz. MP 4/4 was totally sterile while three plants MP 4/3, MP 4/5 and MP 4/31 had only 1% stainability and rest of the three plants MP 4/6, MP 4/7, MP 4/30 had 47.21%, 13.1% and 35.64%, respectively. The mean pollen size varied from 38.81 µm to 33.0 µm and range from 30 µm-48 µm. BC_1 MP 4/10 presented severe case of chromosome stickiness in meiosis. The stickiness prevailed till metaphase I. Chromosomes counting at these stages was not possible. However, after metaphase I stickiness was not observed. 89.28% pollen mother cells showed chromosome stickiness at diakinesis and 78.13% pollen mother cells showed stickiness at metaphase I. Other meiotic abnormalities were also observed. There was a difference between the cell cycle of the two genomes involved in the hybrid. In subsequent stages, one of the genomes reached the poles while the another genome was undergoing sister-chromatids segregation. At diad unequal chromosomes were found. At metaphase II, up to 9 univalents were seen.

IV. H1T2 (*2n* = 35): 4 hybrids were grown in which meiotic studies were carried out in two hybrids. Both had a chromosome constitution of 2n = 35 (Plate 10, picture 15-18).The study showed average chromosomal configuration of $4.68_{I} + 12.96_{II} + 0.56_{III} + 0.59_{IV} + 0.06_{V}$. No hexavalent was observed. Bivalents, as usual, constituted the predominant configurations, with 70.23 and 78.29% chromosomes involved in such configuration. Out of the two plants pentavalent configurations (average 0.12) were also observed. As expected, meiotic abnormalities were also seen. At metaphase I, 7 bigger univalents of pearl millet were invariably seen. Unequal chromosome distribution of chromosomes present at diad (22+13). Triads were also observed. The average chiasma in these 2 hybrids was 21.44 and range was 16 to 31 respectively. One of the two plants

viz. MP 5/26 was male sterile while second plant MP 5/27 had pollen fertility of 29.41 %. The mean pollen size was 40.08 μm and range 33 μm -48 μm.

Backcrosses involving H_2: Since H2 (Hybrid 2) was apomictic in nature only unidirectional cross involving H2 as pollen parent was possible with both T1 and T2 pearl milletparents.

I. T1H2 ($2n = 35$): Out of 48 hybrids, five plants were cytologically analyzed and were found to have chromosome constitution of 2n = 35 (21G + 14S). At diakinesis, the average chromosomal configuration in 115 pollen mother cells was $5.5_{I} + 10.82_{II} + 2.304_{III} + 0.234_{IV}$. The meiotic system, like all other BC_1 plants, showed association from univalents to quadrivalents. Predominant bivalent associations constituted 53.49 to 68.34% chromosomes of the compliment (Plate 10, picture 24-30). Early and late disjunction of chromosomes at metaphase I (up to 11 chromosomes), laggards at anaphase I (up to 9), tripolar anaphase I, unequal chromosome separation and chromosome bridges at diad, presence of micronuclei were some of the meiotic abnormalities observed. The average chiasma in all these 4 hybrids was 19.98 and range was between 13 to 27. MP 6/4 had the highest pollen stainability (14.28%) and MP 6/5 and MP 6/9 were sterile. Mean pollen size was recorded as 38.1 μm and 42 μm in MP 6/4 and MP 6/6 respectively. The size range was recorded as 30 μm -45 μm.

II. T2H2 ($2n = 35$): 32 BC_1 plants were grown out of which four plants of this combination were worked out for detailed cytological analysis (Plate 10, picture 31-40). The average chromosomal configuration in 93 pollen mother cells of 4 hybrids was recorded as $4.69_{I} + 12.47_{II} + 1.43_{III} + 0.268_{IV}$. All the plants had an expected chromosome constitution of 2n = 35 (21G + 14S). Chromosome configurations included quadrivalents, trivalents, bivalent and univalents. Here again predominant configuration were bivalents which accounted for 60.34 to 82.74% chromosomes of the compliment. The bivalent percentage was 71.26 %. MP 4/14 had the highest bivalent frequency of 82.74%, followed by 74.29% in MP 4/15, 67.14% in MP 4/17 and lowest with 60.34% in MP 4/33.

Meiotic abnormalities as expected in hybrids, were observed. Early and late disjunction of chromosomes at metaphase I and II (up to 14 chromosomes), laggards at anaphase I (up to 4), unequal chromosome separation and chromosome bridges at diad and tetrad, presence of micronuclei were observed. In some cases nucleolus appeared at diad stage. Precocious division of chromosomes to chromatids was observed at

metaphase I. Chiasmata frequency per cell was found with an average of 29.35 and range was recorded from 14 to 30.

MP 4/14 had the highest pollen stainability i.e. 47.26% followed by 21.71% in MP 4/33 and 18.78% in MP 4/15. Pollen had the highest value of size mean 43.2 µm and range of 30 µm -51 µm in MP 4/33, size mean of 38.1 µm and range in MP 4/15 and size mean of 36 µm and range from 33 µm -42 µm in MP 4/14. MP 4/17 was male sterile.

F_2 generation: F_2 plants were raised from selfed seeds of H1. Two plants were analyzed cytologically. Both the plants had parental constitution of 2n = 42, however variation in number and type of association was recorded. In one plant only bivalent and univalent association were observed, while in another plant hexavalent associations were observed. Both these plants deviated from F_1 progenitor wherein quadrivalent, trivalent, bivalent and univalent associations were observed. Percentage of chromosomes involved in bivalent configurations varied from 67.38 to 89.19, as against 82.66 of F_1 parent.

F_1 sib: Population was produced by sib mating between H1 and H2 using H1 as female parent. The plants were grown in the field, which were intermediate to both parents in morphology. One sib plant was cytologically analyzed. This plant showed 2n=42, with average chromosomal association $2_I + 20_{II}$. Univalents ranged from 0-6 and ring bivalents from 7-17 and rod from 3-12. Ring bivalents observed with an average of 13.1 while rod with 6.9. The bivalent percentage was 95.24%. The association occurred maximum times was $2_I + 20_{II}$ with 40%. The chiasma frequency was 33.1 and chiasma range was 26-37. Meiotic abnormalities were found. Late disjunction at metaphase I and up to 7 univalents lagging at anaphase I was observed.

Analysis of cytological data of BC_1 plants (pearl millet and *P. squamulatum*):

The Analysis Of Variance (ANOVA) was calculated between groups and within groups of BC_1 plants (H1T1, H1T2, T1H1, T2H1, T2H2 and T1H2).

ANOVA: The F value for trivalents (2.950002) was found to be significant and pentavalents (3.930407) was found to be highly significant. But the F values for univalents, bivalents, quadrivalents, hexavalents, heptavalents and octavalents between the groups was observed to be 1.234255, 1.121649, 0.291834, 0.22638, 0.279686 and

1.234286 respectively which was non-significant. The F-values within groups for all associations were observed as non-significant.

Table A.

ANOVA (univalents)						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	12.85859	5	2.571718	1.234255	0.313442	2.477165
Within Groups	75.01031	36	2.08362			
Total	87.8689	41				

Table B.

ANOVA (bivalents)						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	12.40246	5	2.480493	1.121649	0.366438	2.477165
Within Groups	79.61293	36	2.21147			
Total	92.01539	41				

Table C.

ANOVA (trivalents)						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	5.848126	5	1.169625	2.950002	0.024781	2.477165
Within Groups	14.27338	36	0.396483			
Total	20.12151	41				

Table D.

ANOVA (quadrivalents)						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.253495	5	0.050699	0.291834	0.914353	2.477165
Within Groups	6.254113	36	0.173725			
Total	6.507608	41				

Table E.

ANOVA (pentavalents)						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.006514	5	0.001303	3.930407	0.006035	2.477165
Within Groups	0.011933	36	0.000331			
Total	0.018448	41				

Table F.

ANOVA (hexavalents)						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.005079	5	0.001016	0.22638	0.948651	2.477165
Within Groups	0.161549	36	0.004487			
Total	0.166629	41				

Table G.

ANOVA (heptavalents)						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.005979	5	0.001196	0.279686	0.921221	2.477165
Within Groups	0.153928	36	0.004276			
Total	0.159907	41				

Table H.

ANOVA (octavalents)						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.001429	5	0.000286	1.234286	0.313429	2.477165
Within Groups	0.008333	36	0.000231			
Total	0.009762	41				

Chi^2 Test: This Chi^2 test was analyzed in the F_1 hybrids and BC_1 plants of pearl millet and *P. squamulatum*.

$$\text{Chi}^2 \text{ test} = \frac{(\text{observed value} - \text{expected value})^2}{\text{Expected value}}$$

Observed and expected values of X^2 in F_1 and BC_1 populations of pearl millet x *P. squamulatum*.

The expected X^2 value for F_1 was 21 II. All the values were observed to be non-significant (less than 3.84).

The value of X^2 test were significant (more then 3.84) for trivalents in H1T1, H1T2, T1H1, T2H1 and T2H2 and significant for bivalents in H1T2, T2H1 and T2H2 for the expected chromosomal association of $7_{III} + 7_{II}$ while the X^2 values were non-significant in all crosses for the expected association of $14_{II}+7_I$

Table I: F_1, observed X^2 value

Groups	Observed X^2 value
H1	0.63
H2	0.16
H3	0.12
H4	0.48
H5	0

Table J: BC_1, observed X^2 value.

Groups	III	II
H1T1	4.14	3.67
H1T2	5.92	5.07
T1H1	4.03	3.30
T2H1	4.73	3.96
T2H2	4.43	4.27
T1H1	3.15	2.08

Groups	II	I
H1T1	0.27	0.94
H1T2	0.08	0.77
T1H1	0.34	1.20
T2H1	0.21	0.42
T2H2	0.17	0.76
T1H1	0.72	0.32

4.3.3.1.3. Biochemical studies:

The parents, two F_1 hybrids (H1 and H2) and 28 BC_1 of pearl millet and *P. squamulatum* were studied to estimate the genetic diversity among the hybrids. Three isozyme systems i.e. Superoxide desmutase, Esterase, Peroxidase, were subjected to horizontal starch gel electrophoresis using discontinuous buffer system and native protein was subjected to polyacrylamide gel electrophoresis (PAGE) using discontinuous buffer system. The estimation of the genetic diversity was done by scoring and numbering of the bands on the basis of their relative mobility towards anodal/cathodal ends (Table 4.38, Plate 11).

Banding pattern of different enzymes:

Esterase: Esterase banding pattern revealed the presence of total 21 bands (Table 4.39). The rm value of these bands varied from 0.21 to 0.75. Polymorphism was recorded for all the bands except band no. 11 (rm 0.56). Band no. 4 (rm 0.33) and 12 (rm 0.58) was exhibited only in T1. Similarly band no. 7 (rm 0.4) was present only in BC_1 (MP 5/35, T1H1), band no. 8 (rm 0.43) and 9 (rm 0.45) in hybrid 1(H1). Band no. 2 (rm 0.22) was partely exhibited in the parents, fully expressed in F_1 hybrids and 85.71% BC_1 hybrids (absent in four BC_1 hybrids: MP 4/15, MP 4/14 (T2H2) and MP 2/20, MP 2/21 (T1H2). Band no. 3 (rm 0.3), which was specific to T1and T2, was expressed only in one BC_1 (MP 2/32, T2H1). Band no. 5 (rm 0.37) was partly exhibited in parents, totally absent in F_1 hybrids and BC_1 hybrids of T2H2 and T1H2. Band no. 10 (rm 0.5) was partly exhibited in the parents, present in F_1 hybrids and absent in BC_1 hybrids of T1H1 and T1H2. Band no. 13 (rm 0.61) was partly exhibited in one of the parents (T2) and absent in *P. squamulatum* and F_1 hybrids. Also these two bands were absent in BC_1 reciprocal

crosses, H1T1 and T1H1. Band no. 14 (rm 0.63) was identified only in T2 and two BC_1 hybrids (MP 4/15 and MP 4/12, T2H2). A new band (band no 15 with rm 0.6) which was absent in parents, appeared in BC_1 hybrids in all H1T2, and exhibited only in one plant of T2H2 (MP 4/14). Similarly band no. 16 (rm 0.65) was present in one of the parents viz. T2 and in five BC_1 hybrids (MP 5/8 H1T1, MP 4/30 T2H1, MP 4/3, MP 4/6 and MP 4/31 T2H1). Seven BC_1 plants MP 4/31, MP 4/6 (T2H1), MP 4/15, MP 4/12, MP 2/24 (T2H2), MP 4/10 and MP 2/21 (T1H2) exhibited band no. 17 (rm 0.66). Band no. 18 (rm 0.7) was absent in T2 and few BC_1 hybrids (MP 2/29, MP 5/6 (H1T1), MP 4/30, MP 4/31, MP 4/6 (T2H1), and MP 4/15 (T2H2). Band no. 20 and 21 (rm 0.73 and 0.75 respectively) were exhibited only in *P. squamulatum* and few BC_1 hybrids.

The rm value ranged from 0.21 to 0.75. The number of bands per plant ranged from 2 to 10.

Superoxide Desmutase: The total number of bands identified were 7 in this enzyme (Table 4.40). The number of bands ranged from two to seven with two bands in BC_1 hybrid (MP 2/29) and seven bands in BC_1 hybrid (MP 5/24). Band no. 4 (rm 0.38) was represented minimum times with 6.06%. Band no. 1 (rm 0.32) and 5 (rm 0.74) were the most prominent bands and exhibited in all the parents, F_1 and BC_1 hybrids with 100%. Band no. 6 (rm 0.77) was absent in one of the parents i.e. T1 and three out of 28 BC_1 hybrids (MP 2/29 H1T1, MP 4/31 T2H1, and MP 4/12 T2H2). Band no. 2 (rm 0.33) was partly exhibited in the parents and the hybrids, while band no. 3 (RM 0.36) was absent in T1 and T2 and present in *P. squamulatum* and one of the F_1 hybrids (H2). Band no. 4 (rm 0.38) was an extra band which appeared in only two BC_1 hybrids MP 5/24 (H1T1) and MP 5/39 (T1H1). The H1 hybrid differed H2 in lacking band no. 3 (rm 0.36). Band no. 6 (rm 0.72) was absent in one of the parents T1 and three BC_1 hybrids, MP 2/29 (H1T1), MP 4/31 (T2H1) and MP 4/12 (T2H2). Similarly band no. 7 (rm 0.8) was absent in T1 and seven BC_1 hybrids. The relative mobility of bands ranged from 0.32 to 0.8.

Peroxidase: The study revealed the presence of five bands the slowest band observed at 0.03 rm and the fastest band was observed at 0.21 rm (Table 4.41). The number of bands ranged from 2 – 5. Band no. 1 was absent in T1 and was partly exhibited in BC_1 T1H1, T2H1, and T2H2. band no. 2 (rm 0.04) was absent in H1T2 and partly present in T2H1 (MP 4/30, MP 4/6). Band no. 3 (rm 0.06) was absent in BC_1 (in all H1T1 and in all T1H1 except MP 5/35). Band no 4 (rm 0.18) was exhibited only in *P. squamulatum*. Band no. 5 (rm 0.21) was predominant in all samples except MP 2/32 (T2H1).

Native protein: The study showed the presence of total 16 bands with rm value ranging from 0.06 to 0.61 (Table 4.42). Diversity was recorded for all bands except band no. 7 (rm 0.27). Band no. 1 (rm 0.06) was exhibited only in BC_1 MP 2/29 (H1T1) and band no. 3 (rm 0.1) was present only in H2 each with 3.03%. Band no. 2 (rm 0.08) was present in all the parents and hybrid 1 but absent in hybrid 2. This band was prominent in all H1T1 except one (MP 5/15). Out of six T1H1 plants, only two (MP 5/32 and MP 5/30) and three plants (MP 2/17, MP 4/4 and MP 4/31) out of nine T2H1 plants exhibited band no. 2 (rm 0.08). Band no. 4 and 5 (rm 0.15 and 0.18, respectively) were present in all parents and hybrid 1 while band no. 5 was absent in H2. Band no. 4 was exhibited in three H1T1 plants (MP 5/24, MP 2/29 and MP 5/15) while absent in other two (MP 5/6 and MP 5/8). Similarly this band was present in four T1H1 plants (MP 5/31, MP 5/39, MP 5/32 and MP 5/30) while absent in MP 5/29 and MP 5/35. The presence of this band no. 4 in T2H1 BC_1 plants was 44.44% (four out of nine plants exhibited this band) and completely absent in H1T2, T2H2 and T1H2. Single H1T1 plant (MP 5/24) out of the 5 plants exhibited band no. 5 (rm 0.18) while this band was present in all T1H1 except two plants (MP 5/30 and MP 5/35). Band no. 5 and 6 (rm 0.18 and 0.2) were fully expressed in H1T2 and T2H1 while band no. 5 was partly exhibited in T2H2 while absent in one T1H2 plant (MP 4/10) out of three. Band no. 6 was (rm 0.02) exhibited in *P. squamulatum* IG 98-361, T2 and hybrid 2 and in MP 4/12 (one out of three) Band no. 8 (rm 0.34) and 9 (rm 0.35) were rare bands which exhibited at 9.09%. These bands were exhibited in two T1H1 plants (MP 5/31 and MP 5/39). Band no. 8 was present in MP 5/8 (H1T1) and band no. 9 was present in T1. Band no. 11 (rm 0.37) was partly expressed in the parents and the F_1 hybrids. One plant of each cross, MP 5/15 (H1T1), MP 5/31 (T1H1) and MP 4/6 (T2H1) exhibited this band. All plants of T2H2 and T1H2 showed the presence of this band. Band no. 12 (rm 0.4) was exhibited in T1 and T2 while absent in *P. squamulatum* and both the hybrids. One plant of H1T1 cross (MP 5/8), all plants of T2H1 except MP 4/7, one plant of T2H2 (MP 2/24) and all plants of T1H2 showed the presence of this band. Similarly band no. 13 (rm 0.48) was exhibited in all parents, partly exhibited in F_1 and BC_1 hybrids. One plant of H1T1 (MP 5/6), two plants of T1H1 (MP 5/32 and MP 5/35) lack this band while one plant of T2H1 (MP 4/6) and one plant of T2H2 (MP 2/24) exhibited this band. Band no. 14 (rm 0.5) occurred at 90.91% and BC_1 plants except for three BC_1 plants (MP 5/32 and MP 5/35 T1H1, MP 4/31 T2H1). Band no. 15 (rm 0.52) was exhibited by the parents but lacking

in the F_1 hybrids. It was fully expressed in H1T1 and H1T2 but partly expressed in T1H1. Band no. 15 was absent in two T2H1 plants (MP 2/18 and MP 4/31) out of nine plants and exhibited in one T2H2 plant (MP 4/14). Band no. 16 (rm 0.61) was a rare band which exhibited only in two plants *P. squamulatum* and MP 4/14 (T2H2).

Similarity among interspecific hybrids and parents: The similarity between the plants was estimated using the Jaccard similarity matrix. Dendogram was formed by SAHN agglomerative clustering using the UPGMA algorithm. The dendogram based on 49 bands of three enzyme systems (EST, SOD and POD) and protein native of thirty three plants showed the presence of 5 main clusters which were further divided in total 12 sub-clusters (Fig. 8).

Cluster –1 comprised of 2 BC_1 plants that showed 67% similarity with each other.

Cluster –2 comprised of 8 plants that showed intra-cluster similarity ranging from 53 to 87%. The cluster was further subdivided into 3 sub-clusters. Cluster 2-1 comprised of only one BC_1 plant (33). Cluster 2-2 comprised of two plants (27 and 25). Cluster 2-3 contains five plants (32, 31, 30, 29, and 26) all were BC_1 plants.

Cluster –3 comprised of seven plants, all of which were BC_1 plants that showed 93% to 67% similarity. The cluster was further divided into 2 sub-clusters. Cluster 3-1 comprised of single BC_1 plant (17). Sub-cluster 3-2 comprised of six BC_1 plants (22, 21, 20, 19, 16, and 15) out of which five were of T2H1 and one of H1T2. They showed 76% to 93% similarity among themselves.

Cluster –4 comprised of five plants including one of the parent (14). The cluster was further subdivided to two sub-clusters with 63% to 79% similarity. The sub-cluster 4-1 comprised of two three plants (24, 18, 14) while sub-cluster 4-2 comprised of two BC_1 plants (6, 7).

Cluster –5 was the largest among all the clusters and comprised of 11 plants that showed intra-cluster similarity between 56% and 90%. The cluster was further sub-divided into 4 sub- clusters. Sub-cluster 5-1 was represented by a single plant (3), which appeared to be a bridge between two clusters. Sub-cluster 5-2 again was represented by a single plant (5). Sub-cluster 5-3 comprised of three BC_1 (13, 12, 11) plants with similarity between 74% to 84%. Sub-cluster 5-4 contains six plants (10, 9, 8, 4, 2, and 1) with 71% to 90% similarity.

Grouping of interspecific hybrids (on the basis of EST, SOD and POD isozymes and native protein) of parents among 33 plants of *P. squamulatum* and pearl millet hybrids.

Cluster no.	Sub-cluster no.	Plant no./sample no.*	Accessions
1	1-1	28, 23	MP 4/12 (T2H2), MP 4/31 (T2H1)
2	2-1	33	MP 5/35 (T1H1)
	2-2	27, 25	MP 4/14 (T2H2), H2
	2-3	32, 31, 30, 29, 26	MP 2/21 (T1H2), MP 2/20 (T1H2), MP 4/10 (T1H2), MP 2/24 (T2H2), MP 4/15 (T2H2)
3	3-1	17	MP 2/32 (T2H1)
	3-2	22, 21, 20, 19, 16, 15	MP 4/4 (T2H1), MP 2/17 (T2H1), MP 4/3 (T2H1), MP 4/7 (T2H1), MP 2/18 (T2H1), MP 5/26 (H1T2)
4	4-1	24, 18, 14	MP 4/6 (T2H1), MP 4/30 (T2H1), *P. glaucum* IG 2000-01 (= T2)
	4-2	7, 6	MP 5/8 (H1T1), MP 5/6 (H1T1)
5	5-1	3	*P. glaucum* IG 99-748 (= T1)
	5-2	5	MP 2/29 (H1T1)
	5-3	13, 12, 11	MP 5/30 (T1H1), MP 5/32 (T1H1), MP 5/39 (T1H1)
	5-4	10, 9, 8, 4, 2, 1	MP 5/31 (T1H1), MP 5/29 (T1H1), MP 5/15 (H1T1), MP 5/24 (H1T1), H1, *P. squamulatum* IG 98-360

*Ref. Table 4.39- 4.42

4.3.3.2. Pearl millet x *P. orientale*: F_1 and BC_1 of this interspecific cross previously produced (Zadoo and Singh 1986) and maintained at IGFRI Jhansi were cytologically analyzed and new interspecific crosses were attempted to these already existing hybrids. The new hybrids produced were analyzed cytologically and morphologically. In general these categories of hybrids are denoted as GO (glaucum orientale) hybrids.

4.3.3.2.1. Morphological and cytological observations in GO (glaucum orientale) and GOS (glaucum orientale squamulatum) hybrids:

F_1 (GO) and BC_1 (GO): A single F_1 hybrid plant *(P. glaucum* x *P. orientale)* raised by Zadoo and Singh (1986), was studied which had less hairy and yellow coloured node, yellow leaf base colour, non hairy leaf base and leaf, straight leaf orientation and the leaf colour was observed as green. The awns were varied from yellow to violet in a single spike and the stigma was white and bifid (Table 4.43).

The cytology of this hybrid revealed that number of PMC per anther was lesser than either of the parents. 15 PMC were observed at diakinesis in which 9 chromosomes of *P. orientale* and 7 chromosomes of *P. glaucum* remain unpaired. Condensation difference was also observed between the chromosomes belonging to the two genomes at pachytene. Precocious division was observed, 16, 23, 32 and more than 32 chromosomes in some pollen mother cell at diakinesis were observed. At anaphase I, 3-10 (1-6G +2-7O)* chromosomes at one pole while 3-11 (1-6G +2-7O) at another pole

were observed. Laggards were also observed (0-5G +0-4O). The plant was highly male sterile and partially female fertile.

Three BC_1 plants were studied for morphological attributes. These all were perennial like the female parent, and similar to each other for all the qualitative characters except for the colour of stigma which was found to be white + violet in BC_1 plant (Plant 2 Table 4.43). When these BC_1 were compared to the F_1 hybrid, most of the qualitative characters were observed to be similar except few. The node was hairy in all BC_1 as compared to less hairy node in the F_1 hybrid. The leaf base colour and the leaf orientation was found to be yellow +violet and semi drooping respectively in BC_1 plants while in the F_1, the leaf colour was yellow and the orientation was straight.

Three BC_1 plants had variable values for metric traits except for few traits which were quite similar in all the BC_1 plants like number of leaves, 3rd leaf width, stem girth, number of nodes and internode length (Table 4.44). The F_1 hybrid had high values than the BC_1 plants, for most of the metric traits. Number of tillers, height of main tiller, peduncle length, number of florets per spikelet, flag leaf length and width and number of nodes were higher in the F_1 than the BC_1 plants. However, the other metric traits in F_1 hybrid were observed to be equal or less than the BC_1 plants (Plate 11).

Cytological studies in two BC_1 plants (plant 2 and plant 3 Table 4.45) were carried out and it was observed that these plants contained 2n= 23 chromosome number (Plate 13, 14 and 15). Plant 2 showed highly asynchronous microsporogenesis. The chromosomal configuration at diakinesis in PMC showing normal division was $[0.16G + 3.56O]_I + [6.92G + 2.72O]_{II}$. In PMC showing abnormal meiosis 0-2 univalents and 5-7 bivalents of *P. glaucum*, while 1-8 univalents, 0-5 bivalents, 0-1trivalent and 0-1 quadrivalent of *P. orientale* were observed. At anaphase I, 7G 2O: 7G 7O were found. Micro nucleoli were also observed in mitotic cell. Uninucleate microsporocytes in pachytene were commonly observed but binucleate microsporocyte in pachytene showing two nucleoli and a small bunch of chromosomes which was lying apart from the two nucleoli was also found (Plate 14, picture. 3- 6). Mature pollen grains were observed which were undergoing meiosis I. $6_{II}+2_I$ and $7_{II} + 2_I$ of *P. glaucum* were observed in diakinesis in few cases could be because of cytomixis (Plate 14). The number of *P. orientale* chromosomes varied in the PMC and up to five bivalents of *P. orientale* were observed. Precocious division of *P. glaucum* chromosomes was commonly seen. A cytoplasmic channel was observed between adjacent PMC in all the

stages of meiosis I in most of the cases (Plate 15, picture 1-3, 8 and 9). The PMC showing cytomixis, were at different stages of meiosis I viz. one PMC was at early prophase while the other was undergoing diakinesis, one was at diakinesis while the other showed telophase I (Plate 15, picture 5, 10 and 11). It was also observed that all chromatin material was transferred to the other PMC leaving the former one empty (Plate15, picture 6). In the PMC showing cytomixis, the number of *P. glaucum* chromosomes was observed as expected in both the adjoining PMC while the *P. orientale* chromosomes migrated through the cytoplasmic channel and paired with the homologous (Plate 15, picture 9). $3_{II} + 6_{I}$ of *P. orientale* in one PMC and $2_{II} + 3_{I}$ in the other were also observed (Plate 15, picture 9). On observing the stainability of the pollen grains it was observed that the plant was male sterile.

Unlike plant 2, the BC_1 plant 3 showed normal microsporogenesis. The chromosomal configuration at diakinesis in 25 PMC was $(0.24G + 7.4O)_{I} + (6.88G + 0.8O)_{II}$. The range of glaucum univalents at diakinesis was 0 to 2 and orientale univalents was from 7 to 9, while bivalents of glaucum ranged from 6 to 7 and that of orientale ranged from 1-4. At anaphase I, 7G 3O: 7G 6O chromosomal separation was observed. The mean chiasma frequency recorded was 13.56% and the range of chiasmata was 11-15. The plant was male sterile.

The new hybrids: The new hybrids between BC_1 and tetraploid pearl millet were named as GO hybrids and the hybrids between BC_1 and F_1 hybrid (pearl millet x *P. squamulatum*) were named as GOS hybrids (Fig. 5).

1. BC_1 (GO) x *P. glaucum* (4x): Two accessions of tetraploid pearl millet (T1 and T2) were used in the hybridization with BC_1 (GO) plants. Hybrids were produced only between two BC_1 (plant 3 and plant 1). Three hybrids were produced, one between BC_1 (plant 3) x T1 and two between BC_1 (plant 1) x T2. These hybrids were numbered as hybrid 1, 2 and 3. These three hybrids were similar among themselves for most of the qualitative characters except leaf base and leaf hairiness. Hybrid 1 possessed non-hairy leaf base and leaf, the hybrid 2 had less hairy leaf base and non-hairy leaf and hybrid 3 had hairy leaf base and hairy leaf. All these three hybrids differed for node colour and leaf colour as compared to the male and female parents. It was green and dark green in the hybrids instead of light violet or yellow and green respectively in the parents. The other characters were partially exhibited in the hybrids (Table 4.43). The metric traits

varied in all parents and hybrids except for the number of leaves, which were about 6 in all plants except T2. Out of the three hybrids, the second one had high values for height of main tiller, peduncle length, spike length, number of florets per spikelet, flag leaf length and width, 3rd leaf length and width, stem girth, number of nodes and internode length than the other two hybrids. The other metric traits were quite similar in all the three hybrids (Table 4.44).

The cytological observations of these three hybrids are described below:

Hybrid 1: The BC_1 (GO) plant was crossed with tetraploid *P. glaucum* (2n=28) and this interspecific hybrid was produced. When the plant was compared to the parents for the qualitative traits, it was observed that it differed for few characters. The node colour was green in the hybrid while the parents had yellow node colour. The leaf base and the awn colour were yellow, similar to the male parent instead of yellow +violet in the female parent. The hybrid had dark green leaves instead of green in the parents (Table 4.43, Plate 12).

Quantitative or metric traits were variable in the parents and the hybrids (Table 4.44). Most of the metric traits were of lesser values in the hybrids than the parents. The male parent T1 had the higher values for most of the metric traits. Except number of tillers, which was highest in the female parent (38) followed by the hybrid (4). The hybrid had the least values for height of main tiller, peduncle length, spike length and width, number of nodes and internode length than both of its parents.

On doing cytological analysis, it was observed that the plant contained 2n=37 chromosomes (28G +9O) (Table 4.45, Plate 16). The average chromosomal configuration at diakinesis was found to be as $7.68_{I} + 10.4_{II} + 0.12_{III} + 2.04_{IV}$. At metaphase I, 7-8 univalents of *P. orientale* and 1-2 of *P. glaucum* were clearly observed.1-2 bivalents, one trivalent and 1-4 quadrivalent was found in an average of 6 PMC. At metaphase I, 7-9 univalents of *P. orientale* and 0-2 univalents of *P. glaucum*, 6-12 bivalents, 0-1 trivalent, and 0-4 quadrivalents were observed. The *P. orientale* bivalent was observed at metaphase I. And at anaphase I, at one pole, 12-15 chromosomes of *P. glaucum*, 0-5 chromosomes of *P. orientale*, 0-2 laggards and at another pole, 12-15 chromosomes of *P. glaucum* and 2-7 chromosomes of *P. orientale* were observed. Precocious division of chromosomes to chromatids at anaphase I was also observed in

some cases (Plate 16, picture 6-11). The chiasmata frequency per cell recorded was 24.12% and the range of chiasmata was 20-28. The plant is male sterile as estimated by 0% pollen stainability.

Hybrid 2 and Hybrid 3: Both the hybrid plants were similar for qualitative characters except for leaf base and leaf hairiness which was observed as less hairy in the plant 2 and hairy in the plant 3. The node colour in the hybrids was green instead of light violet and yellow in the male and female parents respectively. Leaf base colour in the hybrids was similar as the male parent T2 while leaf base and leaf hairiness was partially like T2. The hybrids differ from the parents in having dark green leaves instead of green. The awn colour was yellow like the male parent (Table 4.43, Plate 12).

Both the hybrids differ for many characters among themselves. The second hybrid was vigorous for many characters. All the metric traits varied in both the hybrids when compared to the parents (Table 4.44).

Hybrid 2 (2n=37) showed average chromosomal configuration at diakinesis is $6.88_{I} + 12.92_{II} + 0.28_{III} + 0.8_{IV} + 0.04_{VI}$. The hexavalent like association found at diakinesis was actually the secondary association between a quadrivalent and a bivalent (Plate 16, picture 12-15). The chiasmata frequency per cell recorded was 24.56% and the range of chiasmata was 20-28. The plant is male sterile.

Hybrid 3 contained 2n= 37 chromosomes represented by four doses of *P. glaucum* genome and a single dose of *P. orientale* genome. The average chromosomal configuration at diakinesis was $7.2_{I} + 10.44_{II} + 1.16_{III} + 1.36_{IV}$. Interlocking was observed between quadrivalents and trivalents in few cases. 4-7 univalents were observed at metaphase I (Plate 16, picture 16-20). The mean chiasmata frequency per cell recorded was 22.8% and the range of chiasmata was 19-26. The plant contained 2% pollen stainability.

2. BC_1 (GO) x F_1 (pearl millet x *P. squamulatum*): The BC_1 plant 2 was crossed with either of two F_1 hybrids (H1 and H2) between pearl millet and *P. squamulatum.* One hybrid was produced with H1 and five hybrids were produced with H2. All these new hybrids were perennial. The hybrids were similar among themselves for many qualitative characters like yellow and non-hairy leaf base (except hybrid 4) and leaf hairiness, semi drooping and dark green leaves and bifid stigma. The other characters

exhibited partially in all the hybrids. The qualitative and the metric traits of these new hybrids along with their respective parents (Table 4.43 and 4.44) are described below;

BC_1 (GO) x H1 (Hybrid 4): A single plant was produced (hybrid 4)which differed from the parents in having less hairy and green nodes, yellow leaf base colour, straight leaf orientation while the parents had hairy and yellow nodes, yellow + violet leaf base colour and semi-drooping leaf orientation. The hairiness of the leaf base, awn and stigma colour and bifid +trifid stigma were similar to male parent (H1).

The hybrid had high values for the metric traits than the parents. The characters that had high values than the parents were height of main tiller, peduncle length, number of leaves flag leaf and 3rd leaf width, stem girth and internode length. The rest of the traits differed in the parents and the hybrids.

The cytological observations revealed that this was a trispecific hybrid (2n=44) with 3 doses of *P. glaucum* monoploid genome, 2 monoploid doses of *P. squamulatum* genome and a single monoploid dose of *P. orientale* genome (21G+14S+9O). In this plant, disturbed meiosis was observed. In same anther 18.47% of PMC showed pachytene, 19.51% showed diakinesis, 9.06% metaphase I, 9.06 anaphase I, 23.69% dyad, 3.83% metaphase II, 10.1% anaphase II, 3.48% tetrad and 2.09% showed tripolar meiocytes. In some cases no cell plate formation took place and the chromosome appeared on four poles in same microspore viz. development of coenocytic microspores took place. The average chromosomal configuration at diakinesis was $10.24_{I} + 13.88_{II} + 2_{III}$. The dyad and tetrad formation was normal in many PMC and contained equal distribution of chromosomes (Plate 17, picture 5-20). The chiasmata frequency per cell recorded was 20.6% and the range of chiasmata was 14-28. The plant was highly male sterile.

BC_1 (GO) x H2: Five hybrids arising from this cross were numbered as hybrid 5, 6, 7, 8, and 9. These hybrids had similar leaf base colour (yellow) and non-hairy leaf base, non-hairy leaf, semi-drooping and dark green leaves white coloured and bifid stigma. The characters that differed among the hybrids were node hairiness (three hybrids had green and two had yellow nodes), awn colour (three hybrids had yellow and two had yellow + violet awn), and stigma colour (one out of the five hybrids had violet instead of white stigma). On comparing the hybrids with the respective parents, it was found that the two

hybrids (hybrid 7 and 8) had non hairy instead of hairy nodes of the parents. This character was found in one of the parents of H2 (*P. squamulatum*). The other three hybrids (hybrid 4, 5 and 6) had green nodes instead of yellow or violet in the parents respectively. One hybrid (hybrid 4) had stigma colour, that of the male parent, while the other hybrids possessed white stigma (Table 4.43). All these hybrids differed for the metric traits among themselves as well as when compared to the parents (Table 4.44).

The cytological observations of four hybrids (5, 6, 7 and 8) are described below (Table 4.45):

Hybrid 5: This was a trispecific hybrid (2n=44) with 3 doses of *P. glaucum* genome, 2 doses of *P. squamulatum* genome and a single dose of *P. orientale* genome. This plant showed highly asynchronous meiosis (Plate 18). At pachytene there was a difference in condensation of chromosomes of the three genomes. At diakinesis, the chromosomes of three species could be identified on the basis of staining. *P. glaucum* chromosomes were darkly stained, *P. squamulatum* chromosomes were medium stained and *P. orientale* were lightest. On visual observation, the difference between chromosome sizes of the three genomes was found. *P. glaucum* chromosomes were largest, *P. squamulatum* were medium sized while *P. orientale* were the smallest. The anthers containing mature pollen grains also displayed meiocytes still undergoing meiosis. Some meiocytes entered meiosis at the proper time and progressed normally in the cell cycle, giving rise to tetrads, while in remaining PMC, the cell cycle was interrupted for a long period, i.e. until the former cells had developed into pollen grains. An interesting observation in this hybrid was that the later cells underwent meiosis inside a differentiated cell similar to a pollen grain, i.e. the meiocytes were enclosed in the exine wall. The chiasmata frequency per cell recorded was 12.76% and the range of chiasmata was 10-16. The average chromosomal configuration at diakinesis was $18.56_{I} + 12.6_{II} + 0.08_{III}$. Unequal distribution (26: 23) was observed at anaphase I. The plant exhibited 33.53% pollen stainability. Mean pollen size was 44.25 µm and range was 24 µm -54 µm.

Hybrid 6: Unlike hybrid 5 this plant sowed normal divisions in meiocytes (Plate 19, picture 3-10). The average chromosomal configuration at diakinesis was $7.24_{I} + 15.04_{II} + 2.16_{III} + 0.04_{IV}$. The number of univalents varied from 2-12 at metaphase I (2-7 at one

pole and 0-7 at another). Tetrad formation was normal. The chiasmata frequency per cell recorded was 24.08% and the range of chiasmata was 19-29. The plant was male sterile.

Hybrid 7: The hybrid contained 2n= 44 chromosome number (Plate 19, picture 11-20). The average chromosomal configuration at diakinesis was 4.28_{I} + 15_{II} + 1.36_{III} + 1.39_{IV}. Chromosome stickiness present in at least 2 genomes out of the two (could be *P. squamulatum* and *P. orientale*), at diakinesis i.e. condensation difference present between the three genomes. At anaphase 17: 25 chromosomes and upto 4 chromatids were found. Laggards were frequently observed. Tripolar anaphase I was found. Up to 5 lagging chromosomes were found at telophase I. There was an indication of chromosome linkage (inversion bridges) at dyad and tetrad. Pentad and micro pollen were also found along with tetrad. The chiasmata frequency recorded was 27.39% and the range of chiasmata was 23-33. The plant was male sterile.

Hybrid 8: The average chromosomal configuration at diakinesis was 3.32_{I} +13.6_{II} +2.28_{III} +1.68_{IV} . At metaphase I, 5-9 univalents at one pole while 1-5 chromosomes at another pole were observed. Bivalents, trivalents and quadrivalents were also seen at metaphase I. Laggards and unequal distribution was frequently observed at anaphase I (Plate 20, picture 1-12). The chiasmata frequency recorded was 28.56% and the range of chiasmata was 22-33.

Hybrid 10 (81A4 x H2): This hybrid was intermediate to both the parents when compared morphologically. It possessed few characters of the male parent like hairy node and leaf base, and few characters of the female parent like green leaf colour and white stigma. However some characters were intermediate like yellow +violet awns colour in the hybrid. The yellow node colour and non-hairy leaves which were expressed in the hybrid and were absent in both the parents were of *P. squamulatum,* the male parent of H2 (Table 4.43). The hybrid was vigorous than the parents when compared on the basis of the metric traits (Table 4.44).

81A4 X H2 (T1 x *P. squamulatum* MA)

2n= 14 2n=42 (14G+28S)

(14) (7G +14S)

unreduced gamete reduced gamete

2n=35 (21G+14S)

It was expected in this hybrid that an unreduced female gamete (n= 14) was fertilized with a reduced male gamete (n= 21) that resulted in 2n=35 hybrid plant. This was equivalent to BC_1 plant. It showed abnormal meiosis. All meiotic stages (from pachytene to tetrad) were found in the same anther. The average chromosomal configuration at diakinesis (25 PMC) was $3_I + 11.4_{II} + 3.08_{III}$. Secondary association between bivalents was observed. 3-11 univalents were observed at metaphase I (2-7 at one pole while 0-5 at another). Laggards were common and unequal distribution at anaphase I was observed. 13-17 chromosomes at one pole while 16-20 at another pole were observed. 0-7 chromosomes were found as laggards at anaphase I (Plate 20, picture 13-20). The chiasmata frequency per cell recorded was 22% and the range of chiasmata was 18-26. The plant was male sterile.

5. DISCUSSION

Pennisetum glaucum is an important species which is utilized as both food and fodder. The present study on the genus *Pennisetum* is an attempt to know the affinity among various *Pennisetum* species and their relationship with *P. glaucum*. Morphological characters, cytological studies, isozymic variations and interspecific crossability were the measures to estimate the affinity among species. The production of new interspecific hybrids between pearl millet and the wild species was attempted for alien introgressions in pearl millet that can be utilized in its improvement. Also the new interspecific hybrids were studied for genome analysis.

5.1. Cytogenetic characterization of *Pennisetum* species:

Thirty-two accessions of fifteen *Pennisetum* species were studied for their morphological attributes. These species belong to different gene pools and ploidy levels. All accessions of *P. glaucum*, *P. violaceum*, *P. mollisimum* (primary gene pool), all accessions of *P. schweinfurthii* (secondary gene pool) and three accessions of *P. pedicellatum* (tertiary gene pool NATP D-1, IP 21971 and IP 22095) were observed to be annual while all accessions of *P. ramosum* (tertiary gene pool species IP 22180, IP 22137, IP 21935) were biennial and all the accessions of other species belonging to the tertiary gene pool species used in the study were observed to be perennial. On the basis of morphological observations, the species were grouped in six clusters and it was observed that the *P. glaucum* accessions (81B, IG 99-748 or T1, IG 2000-01 or T2) showed maximum distance from *P. ramosum* (Table 4.3). The two species *P. setaceum* and *P. squamulatum* were also observed to share low similarity with *P. ramosum*. However *P. violaceum* accessions (IP 21634 and IP 21532), a primary gene pool species and 3 tertiary gene pool species (*P. ramosum* IP 21935, all accessions of *P. pedicellatum* and *P. polystachyon*) used in the present study were observed closed to the other tertiary gene pool species viz. *P. orientale, P. divisum, P. flassidum, P. hoheneckeri, P. setosum and P. villosum*. *P. glaucum*, *P. violaceum* and *P. mollisimum* were grouped together in cluster 5 as these are closely related. The high similarity between these forms was expected since they belong to the same biological species according to Harlan and de Wet (1971), and intense gene flows have been reported between them (Robert *et al.* 1992). The consistancy of this group is also well supported by the results of cytogenetic and molecular studies (Clegg *et al.* 1984, Chowdhury and Smith 1988, Khalfallah *et al.*

1993, Martel *et al.* 1996). Close relation between the two species *P. pedicellatum* and *P. polystachyon* was found in the present study. This result is congruent with the close relationship observed between these species on the basis of isoenzyme, mitochondrial analysis and ITS phylogeny (Chowdhury and Smith 1988, Lagudah and Hanna 1989, Schmelzer and Renno 1999, Martel *et al.* 2004) as well as with their possible hybridization due to facultative apomictic reproduction (Clayton 1972).

The genus *Pennisetum* is cytologically very heterogeneous and the chromosome number in the species range from $2n$=10 to $2n$=78. In the present study the cytological observation of the cultivated and wild species was done. 27 accessions of the thirteen *Pennisetum* species were studied and the chromosomes in these species ranged from 2n=10 in *P. ramosum* to $2n$=72 in *P. pedicellatum* (Agros 4).

The meiotic studies revealed that chromosome size of species with x=9 and of the species with x=5 and x=7 were different. The species with x=9 had smaller chromosomes as compared with the species with x=5 and x=7. Our study showed similar result as described by Martel *et al.* (2004). The author has suggested that the ancestral characters of the genus are x=9, small chromosomes, an apomictic mode of reproduction and a perennial life cycle. Species with basic chromosome numbers x=5, 7 and 8 appear in the most recently diversion clades, indicating that the genome structure in *Pennisetum* might have evolved towards a reduced chromosome number and an increase chromosome size. The size difference was distinct in species with small basic chromosome number (x=5 and 7) and species with x=9. We can easily differentiate the size of chromosomes of species with x=5, 7 and species with x=9 on visual observations.

The size of meiotic chromosomes was large in *P. ramosum*, *P. glaucum*, *P. violaceum*, *P. mollisimum* and *P. schweinfurthii* as compared to the other species. Brown and Emery (1957) suggested that *P. ramosum* might be an unbalanced diploid or haploid. The present study indicated this species with 2n=10 to be a normal diploid. The diploid accessions of pearl millet (81B, 81A1 and 81A4) showed normal diakinesis as described by Rangaswamy (1935) and Krishnaswamy (1962) and had mostly 7 ring bivalents with two chiasmata each. However, the nucleolar organizing bivalent was generally rod shaped with one chiasma. Triploid *P. setaceum* ($2n$=27) showed modal chromosome association of 9_{II} +9_{I} and revealed the similarity of two of the constituent genomes and the distinctness of the third genome.

Polyploid *Pennisetum* species often do not show strict bivalent pairing at meiosis (Dujardin and Hanna 1984). Although bivalents were observed in species like *P. polystachyon, P. pedicellatum, P. squamulatum*, etc. univalents, trivalents, quadrivalents, and hexavalents also were present.

The cytological observations of *P. squamulatum* accessions (IG 98-360 or MA, IG 98-361 or MB, IG 2000-36 or MC) revealed that the chromosome number was 2n =56. This study support the assumption of Roy *et al.*(2003) and Akiyama *et al.* (2006) that *P. squamulatum* is an octoploid with basic chromosome number x=7 instead of x=9. While earlier reported chromosome number of *P. squamulatum* is 2n =54 and basic chromosome number x=9 (Dujardin and Hanna 1984). Four isozymes were studied in the present work. The Peroxidase enzyme studies also showed that *P. divisum* was closely related to *P. pedicellatum* (IP 22095) and *P. polystachyon. P. flassidum* found close to *P. violaceum* accessions. No successful interspecific hybrids were reported till date between pearl millet and wild species like *P. divisum, P. villosum, P. flassidum, P. pedicellatum* and *P. polystachyon. P. violaceum* is fully compatible with pearl millet and it also showed closer affinity with wild species mentioned above. If these wild species were crossed with *P. violaceum*, the possibility to get interspecific hybrids increases. Later on these interspecific hybrids could be crossed with pearl millet and can be utilized in hybridization programs.

Isozyme analysis of the *Pennisetum* gene pool reveals polymorphic zymograms of EST, SOD and POD. However GDH showed polymorphism for only two species (*P. setosum* and *P. clendenstenum*). The study of esterase enzyme showed that band no. 1 (rm 0.03) and 2 (rm 0.08) were species specific to *P. ramosum*, while band no. 6 (rm 0.19) was specific to *P. squamulatum* (Table 4.11). SOD isozyme showed that band no. 8 (rm 0.9) and band no. 9 (rm 0.92) were specific to *P. squamulatum* (Table 4.12). Study of Peroxidase isozyme showed that anodal band no. 17 (rm 0.92) was specific to two species *P. squamulatum* (IG 2000-36) and *P. polystachyon* (IP 21902) as given in Table 4.13a. These species specific bands could be utilized in the characterization of the species and interspecific hybrids derived from it. On the basis of POD isozymes, little intraspecific variation was observed between various accessions of *P. polystachyon* and one *P. pedicellatum* (NATP D-1) accession shared similarity with *P. polystachyon*. The *P. pedicellatum* octoploid accession (Agros 4) showed high

similarity with other two *P. pedicellatum* accessions (IP 21883 and IP 21890) while quite far from the three other accessions that showed similarity among them selves (IP 21879, IP 21790, IP 21971).

P. divisum (IP 21962) shared similarity with *P. pedicellatum* tetraploid accession (IP 22095). *P. squamulatum* IG 98-360 and IG 98-361 shared approximate 70% similarity with each other while these two were quite far from *P. squamulatum* IG 2000-36 and shared only 20% similarity. The other species showed little intraspecific variation.

On the basis of Peroxidase, Esterase and Superoxide Desmutase isozymes, it was observed that *P. schweinfurthii* shares very little similarity with *P. squamulatum* while according to Martel *et al.* (2004) it is closest to *P. squamulatum*. The grouping roughly matches with the earlier reports (Chowdhury and Smith 1988, Lagudah and Hanna 1989, Khalfallah *et al.* 1993, Martel *et al.* 1996, Clegg *et al.* 1984, Martel *et al.* 2004). Ingham *et al.* (1993), on the basis of rDNA sequences, reported that the three species *P. purpureum P. squamulatum* and *P. glaucum* represent a recent divergence from a common progenitor or a common genome. The genomic distance between groups could prove helpful in predicting the outcome of hybridization in terms of viability and the identification of the hybrids of these enzymes systems.

The study has resulted in grouping of germplasm based on their genetic distance and can be exploited in the genetic improvement programme. The closely related genotypes are expected to cluster together and representative genotypes from a cluster can be used for evaluation under specific ecological situations and stress conditions and for making any hybridization to transfer the traits from some identified sexual genotypes.

5.2. Induction of polyploidy:

The tetraploidization of *Pennisetum* species was attempted to be utilized in the interspecific hybridization programm as the diploid species does not hybridize easily with other *Pennisetum* species with higher ploidy (Dujardin and Hanna 1989). Many successful reports are available where polyploidization was attempted for breaking the ploidy barrier in interspecific hybridization in *Pennisetum* species (Kaushal *et al.* 2003a). Triploid hybrids from crosses between diploid pearl millet and tetraploid napier grass produced high yields of high quality forage but were sterile. The

chromosomes of these sterile F_1 hybrids were doubled using colchicine, but the female amphihexaploid were not desirable forage types as compared to the sterile triploids (Hanna *et al.* 1984, Gonzalez and Hanna 1984).

Synthesis of tetraploids in pearl millet by colchicine treatment was first reported by Krishnaswamy *et al.* (1950). They induced tetraploidy by administering aqueous solution of colchicine in the shoots of young seedlings. Later on, several workers have induced tetraploidy by colchicine treatments (Gill *et al.* 1966, 1970a, Jauhar 1970a, Jha and Sinha 1986). Induced tetraploidy in pearl millet has been utilized to attempt interspecific and intergeneric crosses, for studying genomic relationships and for transferring genetic material from species with higher ploidy status. Koduru and Krishna Rao (1978) reported four autotetraploids in the progeny, chromosome pairing typical of an autotetraploid, all except one showed chromosome pairing typical of an autotetraploid, while the fourth had a high frequency of univalents that might have arisen due to desynapsis.

For chromosome doubling, colchicine was used. It is an alkaloid extracted from the seeds and corm of a plant species viz. *Colchicum autumnale.* It is soluble in water, alcohol and chloroform and it melts at 155° C. The molecular formula of colchicine is $C_{22}H_{25}NO_6$. The effect of colchicine on mitosis was studied for the first time in mice. Its successful use in inducing polyploidy was reported by Blakeslee (1937) and Nebel (1937). Although of very little commercial use, polyploidy could be successfully induced not only in all major diploid crops to produce autopolyploids but also in hybrids between related diploid species to produce amphidiploids.

Commonly aqueous solutions of colchicine are used for inducing polyploidy. The method of application may vary and the solution may be applied to any meristematic tissue including seed, seedlings, growing shoots or buds. Depending upon the treatment and the material to be treated, the concentration of the solution may also need to be varied.

Four chromosome doubling strategies were evaluated for producing tetraploid in four *Pennisetum* species (belonging to different gene pools) for their effective utilization in interspecific hybridization. The four diploid *Pennisetum* species (*P. violaceum*, *P. ramosum*, *P. schweinfurthii* and male sterile lines of *P. glaucum*) and tetraploid pearl millet were given colchicine treatment for induction of tetraploidy and octoploidy respectively. The treatments were given to the seeds, seedlings, and to the

tillers of the species. Colchicine was used at different concentrations of 0.05%, 0.1% and 0.2% and durations 6 hrs, 12 hrs, 18 hrs and 24 hrs. The details of four types of colchicine treatments given were described in section 3.2.3.

The colchicine injection was found most effective in inducing tetraploidy in case of producing wheat doubled haploids from wheat x maize cross (Sood *et al.* 2003). But in the present case, this treatment was found to be ineffective. In both the treatments, the colchicine treatment to seedling and to shoot which were found effective in induction of tetraploidy, the shoot was cut at the tip and treated with different concentration of colchicine.

Total three induced tetraploids were produced in the present study viz. one in *P. schweinfurthii* and two in *P. glaucum* (81A1 and 81A4). For initial screening of the induced tetraploid plants flow-cytometry of leaf samples was adapted. Further screening was done on the basis of observations of stomatal size as the induced tetraploids will have larger stomata as compared with the diploid parent. The length of stomatal guard cells or stomata size in general is known to relate well with ploidy level (Borrino and Powell 1988, Limin and Fowler 1989, Singh and Sethi 1995). Confirmation of the induced tetraploids was done by detailed cytological analysis. Chromosome number of these plants was observed at diakinesis and metaphase I.

Induction of tetraploidy in *P. schweinfurthii* has been attempted in order to facilitate its crossing with induced tetraploid *P. glaucum.* The resultant colchiploid was expected to be fertile, which in turn can facilitate the transfer of desirable traits like disease resistance and bold grain trait from wild *P. schweinfurthii* to cultivated *P. glaucum.*

Morphological observations revealed that the C_0 generation plant of *P. schweinfurthii* was slow growing than the diploid parent. Cytological analysis of *P. schweinfurthii* revealed that the chromosome number was 2n=28. Multivalent association in induced tetraploids is a common phenomenon, because each chromosome is represented four times in the complement. However, in the present case the multivalent associations were far below than expected. In induced tetraploid *P. schweinfurthii*, out of seven possible quadrivalents, only 0-2 quadrivalents per cell were recorded, with an average of 1.35. Out of a complement of 28 chromosomes of tetraploid, on an average 20.6 were represented as bivalents. Trivalent/univalent configurations represented by 2 chromosomes, whereas on an average 5.4 chromosomes

are associated as quadrivalents. The preponderance of bivalents in tetraploids could be due to the preponderance of open bivalents at diploid level. Lower frequency of multivalents configurations is advantageous in the sense that there are fewer disjunctional aberrations resulting into reasonably good fertility as is indicated by 80% pollen stainability of the tetraploid as compared to 96% of diploids.

Reduced fertility is a common phenomenon in induced tetraploids (Gill *et al.* 1969, Jauhar 1970a), which may result from non disjunctional multivalents, lagging univalents and other cytological abnormalities, or even the genetic constitution of the species that may not be amenable to alteration in chromosome number. The tetraploid *P. schweinfurthii,* however, showed non-confirmity with the general trend in induced polyploids in having reduced cell size, revealed by pollen grain size. Range of variability is however, greater in induced tetraploids (30μ-45μ) as compared to diploid (45μ-54μ). It is interesting to note that the smallest pollen grain of diploid matches with the largest in induced tetraploid. Higher range of variability in induced tetraploids is self-explanatory and can be due to abnormal meiosis.

It is well known that there is a threshold value for cell size, beyond which no further increase is possible. It seems that diploid *P. schweinfurthii,* during the course of evolution has already reached a threshold value as is evident from its bold seeds, larger floral parts and pollen grains. Induced tetraploidy as such has had a negative effect as far as these characters are concerned. It may be of interest to mention that besides pollen grains, the seed size of induced polyploids has also shown a reduction in size.

Two more tetraploids of male sterile *P. glaucum* ($81A_1$ and $81A_4$) were formed by the second method of colchicine treatment discussed earlier. Initial screening was done by DAPI flow-cytrometric analysis of the leaf samples. The ratio between diploid and tetraploid plants was 1:2 (1 of diploid and 2 of tetraploid). Morphological observations revealed that the C_0 generation plants in these two induced tetraploids were slow growing and high tillering. C_1 generation plants of *P. glaucum* (81A1 and 81A4) were found to be vigourous, slow growing and late flowering (Plate 5) than the diploid parents.

The tetraploids contained expected 2n=4x=28. Like in *P. schweinfurthii*, these two tetraploids of *P. glaucum* (81A1 and 81A4) also showed low multivalent associations at diakinesis. Out of seven possible quadrivalents, only 0-3 quadrivalents per cell were recorded in both the tetraploids. Out of a complement of 28 chromosomes

of tetraploid, on an average 11.05 and 11.16 are represented bivalents (in 81A1 and 81A4 respectively). Trivalent configurations represented by 2 chromosomes, whereas on an average 0.8 and 0.04 chromosomes were uniivalents (in 81A1 and 81A4 respectively). It was observed in these induced tetraploids that male sterility was maintained in the C_0 generation. The C_0 generation was than crossed with induced tetraploid of 81B (maintainer of 81A1 and 81A4 lines) to get C_1 generation. The success of getting male sterile C_1 generation proves that T1 is a maintainer of these two induced tetraploids of 81A1 and 81A4. These induced tetraploids were male sterile and female fertile as numerous healthy seeds were obtained in C_1 generation and also the pollen stainability was nil. Although some plants were found to be pollen shedder.

This experiment proves that induction of tetraploidy by colchicine treatment does not affect the genes responsible for male sterility or the genes present in the maintainer. Diploid 81B was the maintainer of diploid male sterile lines 81A1 and 81A4 and the induced tetraploid of 81B (T1), in the present study, was proved to be a maintainer of induced tetraploid of 81A1 and 81A4. This proves that induction of colchiploidy does not affect the male fertility or male sterility genes present in the parent plants. It was also observed that the method of shoot treatment and seedling capillary treatment used in the study are the best methods for induction of tetraploidy in the above mentioned accessions of *Pennisetum.*

Induced tetraploid of these two accessions could prove better tool for interspecific hybridization with the wild species having higher ploidy. The colchicine treatment given was not effective in *P. ramosum* (2n=2x=10) and *P. violaceum* (2n=2=14) as no tetraploid could be obtained in these two species. Limited success of earlier crosses at diploid level as well as of *P. glaucum* tetraploid as one of the parents has resulted in sterile hybrids which could not be maintained (Hanna and Dujardin 1986, Nagesh and Subrahmanyam 1996, Marchais and Tostain 1997). Crossing at enhanced ploidy level is expected to enhance crossability and yield fertile amphiploids. The tetraploid of *P. schweinfurthii* reverted to diploid condition in the C_1 generation. Reversion of induced tetraploid to diploid in the subsequent generations is a common phenomenon (Raman *et al.* 1962, Gill *et al.*1969, Subba Rao 1978, Jauhar *et al.*1976). The tetraploids, if successfully raised can be subjected to selection for fertility from C_1 generation onwards for an effective utilization in *Pennisetum* improvement programme.

5.3. Interspecific hybridization:

Out of 485 interspecific crosses attempted in the study, success was observed in 6 cases. In two compatible crosses of diploid pearl millet with *P. violaceum* and *P. mollisimum*, tetraploid pearl millet with *P. squamulatum*, production of new hybrids equivalent to BC_3 in GO crosses and trispecific GOS hybrids. The GOS hybrids are the first ever report.

Diploid male sterile lines of pearl millet on crossing with *P. ramosum*, gave few shrivelled seeds that failed to germinate. Dujardin and Hanna (1989a) and Nagesh and Subrahmanyam (1996) did not get any seed by this cross. Marchais and Tostain (1997) obtained small embryos in tetraploid pearl millet x *P. ramosum* cross that can be rescued but smaller embryos obtained by diploid pearl millet x *P. ramosum* cross could not be rescued.

The attempts to cross pearl millet (diploid and tetraploid) x *P. schweinfurthii* could not produce any seed while in earlier reports hybrids are reported between this cross (Hanna and Dujardin 1986, Nagesh and Subrahmanyam 1996, Marchais and Tostain 1997).

Diploid male sterile lines of pearl millet on crossing with *P. orientale* (IP 21951) gave shrivelled seeds. No germination was observed in these seeds. Dujardin and Hanna (1989) obtained some hybrids between this cross in one year while no hybrid was obtained in the next year. However, Nagesh and Subrahmanyam (1996) could obtained hybrids between this cross.

Male sterile lines of pearl millet (2x) x *P. pedicellatum* (Agros 4) did not gave any seed while 25 seeds were obtained when tetraploid pearl millet (T1) was used as female parent. These seeds also failed to germinate. Tetraploid pearl millet x *P. pedicellatum* (NATP D-1 and IP 22095) also gave shrivelled seeds that failed to germinate. Tetraploid pearl millet x *P. polystachyon* (IP 22102, IP 22109, IP 22121) also gave seeds which failed to germinate. Partial seed development was observed in diploid pearl millet x *P. pedicellatum* or *P. polystachyon* crosses in the study by Dujardin and Hanna (1989a). In all these crosses, where seeds failed to germinate, embryo rescue is suggested to get successful interspecific hybrids. The difference in getting hybrids in one year and no hybrid in next year, and getting hybrids at enhanced ploidy level of one or both the parents suggests that crossing is highly influnced by enviornment , the ploidy level of the parents and varies from accession to accession.

5.3.1. Embryo rescue: In crop plants, embryo culture is being widely atttempted for variety of applications like rescuing incompatible hybrid crosses. The failure of the endosperm to develop, leading to the death of potentially viable embryos, can be overcome by culturing the developing embryo on artificial medium. From the inception of zygote formation through to germination, the occurrence of post-fertilization disorders constitutes a major hurdle to stable hybrid embryo development in wide crosses. The application of tissue culture techniques, particularly in the area of embryo rescue, has had a major impact on the maintenance and development of hybrid embryos from wide crosses. The dynamics of nutritional requirements, osmotic adjustments, and physical conditions such as temperature, light, and gaseous enviornment must be optimized for a successful embryo rescue. Various culture media and techniques have been developed for several crop species (Williams *et al.* 1987). Modifications of embryo culture techniques to generate an osmotic potential gradient during various stages of embryo development have been discussed by Williams (1987).

Dujardin and Hanna (1989a) obtained no hybrids between pearl millet (diploid and tetraploid) and *P. polystachyon* (2n=54) and with *P. pedicellatum* (2n=36) by using conventional crossing techniques. However shrivelled seeds in diploid pearl millet x *P. pedicellatum* or *P. pedicellatum* were observed. Same case was observed in the present study. Shrivelled seeds were obtained on crossing tetraploid pearl millet with hexaploid *P. polystachyon* (IP 22109 and IP 22102) and with octoploid *P. pedicellatum* (Agros 4). But no germination was observed in these seeds. To get successful interspecific hybrids in these two crosses, embryo rescue was attempted and hybrids were obtained in both the crosses. No germination of tetraploid pearl millet x *P. pedicellatum* (2n=4x=36 and 2n=6x=54) was observed. However, hybrids were obtained by embryo rescue of crosses between tetraploid pearl millet with *P. polystachyon* (2n=54) and with *P. pedicellatum* (2n=72) as revealed by the morphology of the plantlets. All these hybrids were lost during sub-culturing after 4-6 months of embryo rescue. The reason could be the high chromosomal imbalance of the hybrids that lead to the death of the plantlets or the culture media needs more balanced nutritional and hormonal supplements. Comeau *et al.* (1988) reported the presence of a maize chromosome segment in wheat plant recovered from a wheat x maize cross. In the progeny of a wheat x pearl millet cross (Ahmad and Comeau 1990), one plant grown to maturity, contained a haploid wheat genome plus a single pearl millet chromosome. The

single pearl millet chromosome appeared to have a reduced affinity for spindle attachment. If observations of this type are confirmed, and their frequency increased, the interspecific crosses involving the wild species may prove to be of agronomic value. Additional treatments of the zygote, before cell division starts, by ionizing radiations may increase the recovery of chromosome fragments from the pollen donor in interspecific crosses as suggested by Laurie and Bennett (1988a, b). A major limitation in this area of study at present appears to be efficient culture techniques to allow development of the embryo in the absence of endosperm.

It appears that ploidy barrier plays an important role in interspecific hybridization in the present study. Dujardin and Hanna (1989a) suggested that embryo rescue in the cross between diploid *P. glaucum* and hexaploid *P. pedicellatum* (2n=54) was possible. Present studies also confirmed that embryo rescue in the cross between tetraploid *P. glaucum* x *P. pedicellatum* (2n=72) and *P. glaucum* x *P. polystachyon* (2n=54) can give successful hybrids.

Probability to obtain fertile hybrids may improve by attempting crosses at enhanced ploidy status of the parents and by ammendments in the culture techniques used in the present study. Prospective fertile amphiploids could be used for further genetical and breeding studies as both the species *P. pedicellatum* and *P. polystachyon* contain important fodder characters that can be introgressed to pearl millet.

5.3.2. *P. violaceum* and *P. mollisimum* crosses:

It was found that diploid male sterile lines of pearl millet when crossed with *P. violaceum* and *P. mollisimum* produced numerous seeds as these are compatible crosses. Perfect bivalent formation in F_1, BC_1 and BC_2 plants of *P. glaucum* x *P. violaceum* and F_1 of *P. glaucum* x *P. mollisimum* suggests that no gross chromosomal changes has occurred during differentiation of these species during evolution. Randomly selected 26 BC_1 plants showed 2n=14 chromosomes with regular meiotic divisions thereby confirming the absence of chromosomal aberrations. However, few F_1 and BC_1 plants of *P. glaucum* x *P. violaceum* cross showed unequal distribution of chromosomes in pollen mitosis and formation of unreduced male gamete formation or restitution nuclei that can be used to produce allotriploids after pollination with either of the parents. The high male fertility in these F_1 and BC_1 plants also suggests absence of any chromosomal abberations. Chromosomal abberations like interchanges etc. if present are usually

associated with semi-sterility of gametes, although in exceptional cases interchanges with high fertility may be observed (Burnham 1956).

All the accessions belonging to the primary gene pool of the genus *Pennisetum* have similar karyotypes. According to Martel *et al.* (1996), the two rDNA probes studied have the same location in all these forms. As the cultivated and wild forms sometimes grow sympatrically and cross-hybridize easily to form fertile hybrids with normal chromosome pairing, the organization of repeated sequences, which represent a large proportion of the genome (54% for the pearl millet; Gupta and Ranjekar 1982), is expected to be quite similar within the gene pool. Their observations of the identical chromosomal location of rDNA in these species is consistent with these expectations. Isozyme studies of wild and cultivated species of the primary gene pool have already revealed a high genetic identity (Pilate –Andre 1992), where *P. violaceum* and *P. mollissimum* are considered to be subspecies (*P. glaucum* ssp, *monodii*). However, these forms are strongly differentiated in their morphology, so some genomic differences do exist and are reflected by mechanisms of gene flow control. Prezygotic reproductive barriers, such as pollen competition, that promote homogametic fertilization have been demonstrated (Sarr *et al.* 1988, Robert *et al.* 1991). Postzygotic reproductive barriers expressed by seed malformation (shrunken endosperm) have also been reported in crosses between wild and cultivated forms of the primary gene pool (Amoukou and Marchais 1993). The high degree of genetic similarity reflects the phylogenetic relationships within the primary gene pool. Pearl millet was domesticated from *P. violaceum sensu lato.* Controlled gene flow helps in maintaining the differentiation between wild and cultivated forms for traits of the domestication syndrome (Pernes 1983).

5.3.3. *P. glaucum* and *P. squamulatum* interspecific hybrids:

P. squamulatum is a wild species that belong to tertiary gene pool of pearl millet (Harlan and de Wet 1971) and contain many desirable traits (discussed earlier) that can be introgressed through interspecific hybridization in pearl millet for its improvement. Two F_1 hybrids were produced between pearl millet and *P. squamulatum* which were named as H1 and H2, were procured from IGFRI Jhansi. These were crossed with tetraploid pearl millet in all possible directions for advancement of generation for introgress the useful traits like perenniality, apomixis, high tillering, etc. of *P. squamulatum* to pearl millet (Fig. 9).

Morphological observations of the F_1 hybrids of *P. glaucum* x *P. squamulatum* and their BC_1 generations showed high variations for all the characters studied. The foliage resembled more closely that of *P. squamulatum.* Variation for many characters like apomixis and sexuality, stigma colour etc. was observed in the F_1 plants shows that at least one of the parents was heterozygous (possibly *P. squamulatum* is heterozygous, pearl millet being maintained by selfing could not be heterozygous). Heterozygous nature of *P. squamulatum* was also reported earlier on the basis of method of reproduction (Dujardin and Hanna 1983). The progeny analysis of the two F_1 hybrids revealed that these seggregated for sexuality and apomixis as one hybrid (H1) was sexual while the other one (H2) was apomictic in mode of reproduction. No off type plants were observed in the progeny of H2. Dujardin and Hanna (1989b) also reported segregation for mode of reproduction in the F_1 hybrids of *P. glaucum* x *P. squamulatum* cross. In BC_1 plants segregation was observed for perenniality, high tillering, etc. All BC_1 progenies were intermediate to the interspecific hybrids and pearl millet in their plant characterstics. They had wide range of variation in their vegetative and inflorescence characteristics (Plate 8, picture 2-8). Most of them resembled *P. squamulatum* and the F_1 hybrid in perennial growth habit. The plants having desirable characters can be selected easily and backcrossed with pearl millet for further advancement of generations and for pearl millet improvement. The BC_1 plants were grouped in three catagories on the basis of fertility. 20 plants were found to be male and female fertile, which is a good sign for further advancement of generation as crosses can be attempted in both the directions. 5 plants were male sterile but female fertile and 20 plants were found to be self incompatible but female fertile. Morphological data supports that introgression of all the useful characters (disscussed earlier) in F_1 hybrids as well as in BC_1 plants took place. The hybrids with interogressed desirable traits are being selected and are subjected for further improvement of pearl millet.

A perusal of Table 1.1 reveals that the genus *Pennisetum* is a polybasic genus and different species are based on x = 5, 7, 8 and 9. *Pennisetum glaucum* the species under study is diploid based on x = 7 (2n = 14), whereas the other species involved in present study *P. squamulatum* Fresen. was reported to have 2n = 54 chromosomes (Patil *et al.* 1961, Krishnaswami and Thulasidas 1962, Rangaswamy 1972) which prompted the earlier worker to place it in x = 9 group and has been referred as a hexaploid (2n = 6x = 54). But recent discovery of cytotypes with 2n = 56 (Roy *et al.*

2003, Akiyama *et al.* 2006 and Kaushal *et al.* 2007) and its easy crossability with *P. glaucum* has led to a rethinking about the status of *P. squamulatum.* It is now presumed that *P. squamulatum* is an octoploid (2n = 8x = 56) based on x = 7.

All attempts to cross diploid *P. glaucum* with *P. squamulatum*, including embryo rescue did not yield any hybrids, probably due to ploidy barrier effecting embryo-endosperm ratio. However the hybrids were easily raised once the chromosome number of *P. glaucum* was doubled to 2n = (4x) = 28 through colchicine application. However, reduce quadrivalent frequency and a corresponding increase in bivalent frequency has been observed in T2, a tetraploid acquired from U.S.D.A. The reduced quadrivalent frequency could be due to advanced generation of T2, as compared to T1. Such a phenomenon is well known in induced polyploids, with a tendency of reduced multivalent frequency and increased in fertility in advancing selfed generations. Besides many other examples such a trend has been observed in Brassica (Swaminathan and Sulbha 1959) and Oats (Kushwaha *et al.* 2004).

Tetraploid *P. glaucum* was crossed with *P. squamulatum* and the F_1 hybrids were grown which possess high tillering and perennial growth habit like the male parent. The F_1 hybrids were morphologically similar to their corresponding male parents. Five F_1 hybrids were studied and these hybrids segregated for their sexual and apomictic character. The meiotic studies of all these five F_1 hybrids revealed that the chromosome number was 2n = 42 (14G + 28S). No variable chromosome number in these hybrids shows that only normal gametes (n = 14) from *P. glaucum* and (n = 28) from *P. squamulatum* were involved in the hybridization and the pollen with altered gametic number were nonviable. This reveals that imbalance gametes were nonfunctional or not involved in the hybridization.

The hybrids and BC_1 plants obtained in the present study were different than the hybrids and BC_1 plants obtained by Dujardin and Hanna (1985a) as the *P. squamulatum* accession used by them contained 2n=6x=54 chromosome number and the tetraploid pearl millet accession (T2) was not as much fertile as T1 used in this study. The F_1 hybrids produced by them, contained 2n=41 chromosomes and BC_1 contained 2n=32 to 2n=39. The pollen stainability ranged upto 6.6%.

In the present study, BC_1 hybrids were produced in all possible directions showed an expected chromosome number of 2n =35 and the pollen stainability was quite higher (upto 47.2 %) than obtained by Dujardin and Hanna (1985a). At diakinesis, no

much difference in chromosome association in reciprocal crosses was observed. However the range of chromosome associated as bivalents in H1T1 was 54.11% to 83.43% which was quite similar to T1H1 with 56.74% to 80.86%. Little difference was observed in the reciprocal crosses H1T2 and T2H1. The percentage of chromosomes involved as bivalent in H1T2 ranged from 70.23% to 78.29% while the range was varied in T2H1 with 47.77% to 82.86%.

The multivalent formation higher than the expected one (quadrivalents and pentavalents in BC_1 hybrids) could be because of two reasons

1. Residual homology was present between at least one pair of chromosome of *P. squamulatum* and *P. glaucum* (Dujardin and Hanna 1985b).
2. At least two bivalent have been observed in haploid pearl millet which was generally considered to be a result from residual homology (Person 1955).

Residual homology in chromosomes of pearl millet, *P. purpureum* and *P. squamulatum* is also reported by Dujardin and Hanna (1985b) in trispecific hybrid which was obtained by crossing *P. glaucum-P. purpureum* hexaploid (2n=42) with *P. squamulatum* (2n=54). This trispecific hybrid possessed 2n=48 chromosomes. These hybrids were also subjected for production of BC_1 plants.

The meiotic chromosome behavior was quite irregular in all hybrids and highly irregular in the successive generation studied. Meiotic abnormalities were those common to polyploids and in hybrids i.e. multivalent chromosome association at diakinesis, irregular chromosomes segregation leading to micronuclei formation in the tetrad stage and disturbance in the embryo-endosperm balance number. Decreased fertility in BC_1 plants is due largely to increased meiotic irregularities resulting in unbalanced gametes. Several BC_1 progenies shed enough viable pollen to continue gene transfer and develop BC_2 plants. Further elimination of *P. squamulatum* chromosomes could improve male fertility in future backcross generations.

Double cross hybrids (2n=42) produced by crossing a *P. glaucum* x *P. purpureum* amphiploid with a *P. glaucum* x *P. squamulatum* interspecific hybrid were backcrossed to tetraploid pearl millet (2n=28) for transferring gene(s) of apomixis from *P. squamulatum* to pearl millet by Dujardin and Hanna (1989b). In both these studies by Dujardin and Hanna, BC_1 produced were different from BC_1 produced in the present study in having *P. purpureum* genome. *P. purpureum* was used as a bridging species and along with the characters of *P. squamulatum*; characters were also transferred from

P. purpureum to pearl millet. Another difference was that the *P. squamulatum* accession contained 2n=54 chromosome number. The BC_1 produced in the present study contained only pearl millet and *P. squamulatum* genomes and the *P. squamulatum* accession contained 2n=56 chromosome number.

Both *P. glaucum* and *P. squamulatum* were grouped in the same cluster on the basis of isozyme and native protein studies. This shows that both are closely related. No significant reciprocal cross difference was observed in the BC_1 plants.

On the basis of Super oxide desmutase, band no. 3 (rm 0.36) was observed in *P. squamulatum* while absent in pearl millet and polymorphism for this band was recorded in the two F_1 hybrids (H1 and H2) suggesting segregation in F_1 hybrids. Band no. 4 (rm 0.38) was a new band that appeared only in two BC_1 plants, one plant belongs to H1T1 group while the other belongs to T1H1 group. These two plants were self-incompatible and the new band that appeared could be responsible for the self incompatibility in these two BC_1 plants. Esterase banding pattern revealed that band no. 7 (rm 0.41) was a new band which appeared only in one BC_1 plant belonging to T1H1 group. Band no. 15 (rm 0.64) was also a new band which was absent in the parents but appeared in BC_1 (H1T2) and exhibited in only one BC_1 plant of T2H2. Peroxidase isozyme showed presence of band no. 4 (rm 0.18) only in male parent *P. squamulatum* and was absent in tetraploid pearl millet and F_1 and BC_1 hybrids.

5.3.4. *P. squamulatum*: a hexaploid or an octoploid?

The three accessions of *P. squamulatum* (IG 98-360, IG 98-361 and IG 2000-36) used in the present study contained 2n=56 chromosomes as revealed by the meiotic studies. Earlier it was thought that it is 2n=6x=54+2 with basic chromosome number x=9 (Raman *et al.* 1959, Krishnaswami and Thulasidas 1962, Sisodia 1970, Dujardin and Hanna 1989a, Marchais and Tostain 1997). But the detailed meiotic study of these accessions and their F_1 and BC_1 hybrids with pearl millet pointed towards their octoploid nature with basic chromosome number x=7. The absence of multivalents, such as hexavalents and pentavalents as well as the presence of quadrivalents supports the theory of *P. squamulatum* being an octoploid with basic chromosome no. x =7 (Roy *et al.* 2003 and Akiyama *et al.* 2006). The bivalent frequency was quite high in these hybrids. The perfect bivalent formation in one of the F_1 hybrids (MP 2/9) and more than 83 % bivalent formation in rest of the F_1 hybrids also supports the presumption of Roy *et al.* (2003) and Akiyama *et al.* (2006). However the presence of univalent and trivalents

suggest limited residual homology between one or more chromosomes of *P. glaucum* and *P. squamulatum.* Residual homology in the two genomes was also reported earlier (Dujardin and Hanna 1983). However, this presumption requires detailed genetic analysis. Dissimilar genomic interspecific hybrids with this cytotype may yield information on its possible genomic status by reducing the ploidy level of *P. squamulatum* genome in hybrids where only half of the chromosome set is transferred. Further, since *Pennisetum* is a polybasic genus with base numbers of x=5, 7, 8 and 9, a detailed cytological assay of diverse germplasm of *P. squamulatum* is warranted, to have an insight on the frequency of cytotypes with 2n=54 and/or 2n=56, which will help in understanding the nature and extent of polyploidy in the species.

The few strong reasons that points towards x=7 basic number and octoploid nature of *P. squamulatum* are:

1. All *Pennisetum* species with x=7 cross easily with pearl millet (*P. violaceum*, *P. mollisimum*, *P. purpureum*) except *P. schweinfurthii* which belongs to secondary genepool of pearl millet and does not cross easily with pearl millet. No other species of tertiary gene pool have crossability as good as *P. squamulatum* with tetraploid pearl millet. Although hybrids of pearl millet were reported with *P. orientale*, *P. setaceum* (Patil and Singh 1964, Hanna 1979) but their crossability was very low and the hybrids produced were highly sterile.
2. Roy *et al.* (2003) reported a new cytotype of *P. squamulatum* with 2n=56. Assuming this species with a basic chromosome number x=9, as universally followed, this plant should be containing two extra chromosomes with constitution 2n=6x+2=56. They suggested another possibility of higher ploidy status than hexaploid of this species. They further added that this species could be an octoploid (2n=8x=56) with a basic chromosome number x=7.
3. Martel *et al.* (2004), on the basis of ITS phylogeny, showed that *P. squamulatum* is closest to *P. schweinfurthii* (2n=14, x=7) and than to *P. purpureum* and *P. glaucum.* All these species have x=7 basic chromosome number. Possibility of x=7 basic chromosome number of *P. squamulatum.*
4. According to Akiyama *et al.* (2006), one accession of *P. squamulatum* that had shown a chromosome number of 2n = 56 instead of 2n=54. In order to determine if there was a variation among accessions, they counted the number of chromosomes in 5 accessions of *P. squamulatum* using centromeric and 18S-5.8S-26S rDNA probes

as molecular cytological markers. The results showed that *P. squamulatum* is most likely octoploid with a basic chromosome number of 7 ($2n = 8x = 56$) and may belong to the secondary gene pool of *Pennisetum*.

5. Cytological investigations of F_1 hybrids of pearl millet x *P. squamulatum* (2n=56) cross suggested $2n=8x$ nature of *P. squamulatum* (Kaushal *et al.* 2007).

All these studies are strong enough to prove the basic chromosome number and ploidy status of *P. squamulatum* as suggested in this study. Further, by reducing the ploidy level of *P. squamulatum* genome in hybrids where only half of the chromosome set is transferred, the genomic status of *P. squamulatum* can be confirmed.

5.3.5. New interspecific hybrids:

F_1 hybrid of *P. glaucum* (2n=14) and *P. orientale* (2n=18) and BC_1 plants of this F_1 (F_1 x *P. glaucum* 2n=14) were studied morphologically and cytologically and were used in interspecific hybridization for further advancement of generations. These hybrids were named as GO (glaucum orientale) hybrids. Interspecific hybridization was attempted in all possible directions with pearl millet (diploid and tetraploid), with other wild species, as well as hybrids of these species. No hybrids were obtained involving F_1 (GO) as male parent. It's being highly male sterile could not be used as male parent. Nine new hybrids were obtained when BC_1 (GO) were used as female parent. Out of these nine hybrids, three hybrids were produced between BC_1 (GO) and *P. glaucum* (2n=4x=28). These were equivalent to BC_3 hybrids and contained 2n=37 chromosome number. Six new hybrids were obtained (containing 2n=44), between BC_1 (GO) x F_1 (*P. squamulatum* (2n=56) x *P. glaucum* (2n=28)). These new hybrids were named as GOS hybrids as these contained three different genomes of glaucum, orientale, and squamulatum (Fig. 10). The detailed morphology and cytology of all these F_1 and BC_1 (GO) hybrids and the new hybrids produced is discussed below:

5.3.5.1. F_1 and BC_1 (GO) hybrids: Interspecific F_1 hybrid involving *P. glaucum* (2n=14) and diploid cytotype of *P. orientale* (2n=18) was successfully raised by Patil and Singh (1964). This hybrid was sterile with a perennial growth habit; it exhibited considerable heterosis over the *P. orientale* parent (Singh, 1970)) and could be maintained and multiplied by vegetative slips; it showed 2n=16 chromosomes (Patil and Singh 1964). In order to improve forage yield and quality, the F_1 was backcrossed two times to *P. glaucum* using the latter as pollen parent and cytological analysis of BC_1 and

BC_2 plants from PMC was studied by Zadoo and Singh (1986). These F_1 and BC_1 plants had the following characters (Patil and Singh 1964, Singh 1970, Zadoo and Singh 1986):

1. Perennial growth habit (both parents were annual).
2. Exhibited considerable heterosis over *P. orientale* parent.
3. Could be multiplied by vegetative slips.
4. F_1 showed 2n=16 chromosomes and BC_1 showed 2n=23 chromosomes (unreduced female gamete formation in F_1 was taking place).
5. Male sterile and partial female fertile.

The phenomenon of unreduced egg formation by F_1 and BC_1 has also been described in Zea x *Tripsacum* hybrids using *Tripsacum* with 2n = 36 chromosomes (Harlan and de Wet 1975). The F_1 was found to have 2n = 28, with 18 *Tripsacum* and 10 maize chromosomes, On backcrossing to maize, only unreduced eggs functioned, resulting in all BC_1 plants with 2n=38 (18 'T' + 20 'Z') chromosomes. The production of unreduced eggs is to a large extent under genetic control. In *Zea mays* the gene *'el'* has been found to increase the frequency of unreduced eggs (Rhoades 1956). A case of diploid nucellar embryo sacs functioning sexually has been reported in the *Bothriochloa-Dichanthium* complex (Harlan *et al.* 1964).

The occurrence of a wide array of euploid chromosomal races in *P. orientale* other than the diploid 2n = 18, e.g. 2n = 27, 36, 45 and 54, suggests the operation of a genetic mechanism for the production of unreduced gametes, expressed in hybrid background. Such mechanism for unreduced female gamete formation in *Pennisetum* and its expression in interspecific hybrids as exemplified by the present study may help in explaining the origin of higher polyploids in this genus. Interspecific hybridization involving these F_1 and BC_1 plants was carried out for further advancedment of the generation and futher studies of introgression of useful characters in pearl millet. The cytological analysis of F_1 and selected BC_1 plants showed disturbance in microsporogenesis which were common to interspecific hybrids.

Asynchronous meiosis in F_1 hybrid was observed and preferential migration of *P. orientale* chromosomes through cytomixis at an early stage of microsporogenesis was observed in BC_1 plants of interspecific hybrids between *P. glaucum* x *P. orientale*.

The development of microsporogenesis from early prophase to the tetrad stage was examined to find out the way and the extent to which cytomixis takes place in

F_1 and BC_1 (GO) hybrids. Meiosis was found to be highly asynchronous in many PMC. In the first normal meiotic division, 14 bivalents of *P. glaucum* and 9 univalents of *P. orientale* were found. But the later stages were highly asynchronous. Occurrence of cytomixis during leptotene to pachytene stages of first meiotic prophase was observed (Plate 15, picture 7-12).

The chromatin/chromosome migration preferentially occurred between 2 PMC (Plate 15 and 17) although in some cases 3-5 meiocytes were found to be involved in cytomixis (Plate 17). Cytomixis occurred through cytoplasmic connections consisted of channels of varying breadth that were sufficiently large to permit to the migration of the chromatin/chromosomes among adjacent meiocytes at same phase of meiosis (Plate 17, picture 3, 8 and 9).

The migration of chromatin/chromosomes in PMC involved in cytomixis was usually unidirectional with a donor cell and a recipient cell (Plate 17, picture 6). The existance of cytoplasmic channels between adjoining PMC permitting the transfer of chromatin/chromosomes has been confirmed by several workers (Heslop-Harrison 1966, Risueno *et al.* 1968, Whelan 1974,). Narain (1980) suggested that the amount of material extruded from one cell to another, viz. either a short strand of chromatin or whole chromosomes, could depend on the nature and number of protoplasmic connections of the affected cell. The significance of cytomixis has been variously assessed. It has been considered by some workers as a mechanism of evolutionary importance of plants due to its possible role in the origin of aneuploids and polyploids (Sarvella 1958, Omara 1976, Zeng *et al.* 1987, Falis tocco *et al.*1995). For others, it represents just an unfavourable phenomenon with deleterious effect on fertility (Marechal 1963).

The factors responsible for cytomixis are rather ambiguous. Some possible causes attributed to cytomixis are effect of fixation (Woodworth 1931, Jacob 1941, Linnert 1955, Takats 1959, Gottshalk 1970), mechanical injury (Sarvella 1958), pathological conditions (Bobak and Herich 1978, Morisset 1978), temperature anomalies (Basavaiah and Murthy 1987, Soman and Bhavannandan 1993), polyploid level (Verma et al. 1984), hybrid condition (Narain 1979, Yen *et al.* 1993), cell response as a consequence of pesticides and antibiotic dosages (Kumar and Sinha 1991), abnormal genetic behavior due to treatment with chemical mutagen (Kumar and Srivastava 2001, Kumar and Sharma 2002), crop culture condition (Pierozzi and Benatti Jr. 1998), failure of cell wall formation at premeiotic mitosis (Gates and Rees 1921, Beadle 1932, Kamra

1960), and genetically controlled behaviour (Brown and Bertke 1974, De Mantu and Sharma 1983). The cytomictic condition could be one of the reasons for high male sterility in this hybrid. However, cytomixis alone can not account for the higher pollen sterility observed in the present case. It seems possible that genetic factors might have also contributed to the production of sterile pollen. Cytomixis could prove significant in production of aneuploids and polyploids in this hybrid which can be utilized in further breeding programm for pearl millet improvement. Another reason for high male sterility in these hybrids might be due to the utilization of male sterile line as female parent in F_1 crosses.

In one of the BC_1 plant, highly abnormal microsporogenesis was observed (Plate 16 and 17). The *P. orientale* and the *P. glaucum* genomes did not show same meiotic rhythm. The two genomes of *P. glaucum* and *P. orientale* were observed to follow their own cell cycle and therefore asynchronous microsporogenesis was observed along with normal microsporogenesis. The PMC in Plate 16 (picture 3-6) show a binucleate microsporocyte in pachytene. The two genomes were observed in separate groups in the same microsporocyte.

- Precocious division of *P. glaucum* chromosomes was commonly seen.
- Could be because of preferential and selective migration of *P. orientale* chromosomes that migrated at early prophase through cytomixis and paired with their homologous.

5.3.5.2. GOS hybrids: The GOS hybrids have morphological characters of all the three species involved in crossing viz. *P. glaucum*, *P. orientale* and *P. squamulatum*. Genome relationships in polyploid hybrids have traditionally been determined by meiotic analysis involving an accurate study of chromosome pairing. The new hybrids produced in the present study were initially confirmed by their intermediate morphology than both the parents and than by cytological observations. The first evidence of genomic affinity between *P. glaucum*, *P. orientale* and *P. squamulatum* used as parental material in these hybrids was subjective and presence of nine smaller univalents (or smaller univalents and bivalents) of *P. orientale* at diakinesis or metaphase I indicate that the three genomes are non homologous. There is a difference in the chromosome size and staining of the chromosomes of the three species (Plate 20, picture 3-8). Pearl millet chromosomes were largest and darkly stained, *P. squamulatum* chromosomes were

medium sized and medium stained while *P. orientale* chromosomes were smallest and lightest stained and therefore chromosomes of the three species can be identified at diakinesis. The staining difference of the three genomes can be seen from early prophase to meiosis II.

Male meiosis is generally synchronous in higher plants. However, it is not clear how this synchrony is achieved, nor is it known whether the synchrony is linked to the regulation of cell-cycle progression (Magnard *et al.* 2001). The regulation of the cell cycle is still not well understood, and a powerful tool for gaining an understanding of this regulation is the development of mutation that affects cell-cycle synchrony. In some BC_1 plants and the newly produced GO and GOS hybrids, asynchronous microsporogenesis was observed. Studies performed in most of the BC_1 plants have shown synchronous microsporogenesis at any given stage of anther development while some BC_1 have shown asynchrony in cell division as despite showing the same enviornment inside the anther, not all of the cells received the signal to enter meiosis at same time.

Similar type of asynchrony in meiosis was observed in an interspecific hybrid of *Brachiaria ruziziensis* and *B. brizantha* by Pascotto *et al.* (2004) and an independent genetic control for meiosis synchrony and meiotic stages was suggested. Molecular data has also suggested that each divisional step of meiosis is controlled by a very conservative set of genes (Bhatt *et al.* 2001).

In the present case, pollen stainability was observed to be nil but partial female fertility was observed. When these BC_1 plants were crossed using pearl millet (T1 and T2) pollen, three interspecific hybrids were produced with 2n=37 chromosome number, indicating formation of unreduced female gametes (23 chromosomes from BC_1 and 14 from pearl millet). Zadoo and Singh (1986) also produced BC_2 plants (2n=30) by crossing BC_1 and diploid pearl millet (2n=14). They also reported the formation of unreduced female gametes of BC_1.

In hybrid 4 (BC_1 x H_1), irregular chromosome segregation occurred in most meiotic divisions (Plate 17). Absence of cytokinesis after telophase II could be the cause of male sterility in this hybrid. Instead of the typical tetrads of microspores, four nucleate coenocytic microspores were formed. Defects in cytokinesis following telophase II have been reported in ms1 (Brim and Young 1971, Albertsen and Palmer 1979) and ms4 (Delannay and Palmer 1982, Graybosch and Palmer 1985) male sterile

soybean mutants (*Glycine max*). Sterility of ms1 mutants was caused by a failure of cytokinesis after telophase II.

The abnormalities observed in the present hybrid were different from the abnormalities observed in the parents. The mechanism for unreduced female gamete formation in *Pennisetum* and its expression in interspecific hybrids as exemplified by the present study may help in explaining the origin of higher polyploids in this genus.

The new GO hybrids (BC_1 x tetraploid pearl millet) segregated for perenniality as one out of the three hybrids turned to be perennial and the other two were annual. There is very less or no pairing between the three genomes. Partial homology was reported between only one chromosome of *P. squamulatum* and *P. glaucum* and between one chromosome of *P. orientale* and *P. glaucum* (references disscussed earlier). This shows that the three genomes are non-homologous to each other. In BC_1 (GO), that showed normal meiosis, the 14 *P. glaucum* chromosomes invariably associated as bivalents and *P. orientale* chromosomes remained as one bivalent plus seven univalents or nine univalents. Similar condition of *P. orientale* was observed in new hybrids equivalent to BC_3 (BC_1 (GO) x tetraploid *P. glaucum*) where *P. orientale* chromosomes remained mostly as one bivalent plus seven univalents or nine univalents with exception of two to eight univalents in few cases (more than nine univalents present in the data contained few univalents of *P. glaucum*). The *P. glaucum* and *P. orientale* chromosomes can be easily distingiushed on the basis of larger size of *P. glaucum* chromosomes and smaller size of *P. orientale* chromosomes. No pairing between these two genomes shows that the two genomes are non-homologous.

All the GOS hybrids produced are perennial and high tillering. The genome of *P. squamulatum* has apomict character while *P. orientale* and *P. glaucum* were sexual. These hybrids contained three genomes in common background and it was highly interesting to know how these three genomes behave in the common background. Hybrid 5 contained high frequency of univalents and upto 28 univalents were observed could be because of desynapsis (Plate 18). In other five hybrids upto 18 univalents were observed. The presence of quadrivalents could be due to partial homology between one chromosome of *P. glaucum* and *P. squamulatum*. Trivalents were expected in these hybrids as these contained 21 chromosomes of pearl millet that can be found as 7 trivalents. But upto 4 quadrivalents in these hybrids were found, this suggests that there is partial homology present between more than one chromosomes of *P. glaucum* and

P. squamulatum. Multivalents higher than expected were also observed in the BC_1 plants of interspecific cross of *P. glaucum* and *P. squamulatum* in the present study that shows partial homology between more than one chromosome of the two species. *P. orientale* chromosomes are non-homologous to *P. glaucum*, this is discussed earlier in this section but, the presence of less than seven univalents in GOS hybrids (two orientale chromosomes involved in bivalent foermation due to partial homology) suggests that homology/homeology present between at least five chromosomes of *P. orientale* and *P. squamulatum.* If so, it is possible that the smallest chromosomes of *P. squamulatum* could have size similar to larger chromosomes of *P. orientale* and therefore the staining and size difference present between these two genomes could roughly differentiate between them only if these were present as univalents as discussed earlier. On the basis of all these results it is suggested that *P. squamulatum* has genomic constitution of SSSSSSSS and *P. orientale* has genomic constitution of SS'. So far no interspecific hybrid was attempted between *P. squamulatum* and *P. orientale* and it is suggested that in future studies the interspecific hybrid between *P. squamulatum* and *P. orientale* could be attempted and possibilities to get successful hybrids are there. The interspecific hybrid of these two species could give valuable information about the genomic constitution of the two species which could be utilized in future breeding programms.

The production of these new trispecific GOS hybrids and presence of at least one bivalent of *P. orientale* in F_1 and BC_1 hybrids (GO) also questions on the basic chromosome number of *P. orientale* that wheather it is x=9 which is reported earlier or x=7 as in case of *P. squamulatum*. The presence of some homology between *P. squamulatum* and *P. orientale* chromosomes as revealed by the study and production of interspecific hybrid of *P. glaucum* and *P. orientale* at diploid level also supports that basic chromosome number of *P. orientale* could be x=7 instead of x=9.

These newly synthesized hybrids are vigorous and introgression of characters like perenniality in pearl millet was observed. By successive crossing with pearl millet and selection for the useful traits, these hybrids may prove as a better tool for pearl millet improvement.

It would be very interesting to know that how the three different genomes behave in a common background in further generations. These newly produced interspecific hybrids are subjected to be utilized in the alien introgressions in pearl millet for its improvement and for cytogenetical studies.

6. Conclusions

Following are the salient findings of the present work:

1. Sequential reduction of chromosome complement in interspecific hybrids (F_1, BC_1, Sibs and F_2) between tetraploid pearl millet ($2n=4x=28$) and *P. squamulatum* confirmed *P. squamulatum* as an octoploid at with $2n=8x$ status, and $x=7$ alike pearl millet.
2. Induced tetraploids in male sterile diploid pearl millet lines (with A1 and A4 cytoplasm) were successfully produced.
3. BC_1 derived from pearl millet x *P. squamulatum* cross in this study offer potential for production of MAALs/DAALs, and many desirable characters like perenniality, high tillering, multi cut, apomixis were introgressed for pearl millet improvement.
4. Restricted pairing between chromosomes of pearl millet and *P. squamulatum* revealed different genomic status of the two species, however, homeology for at least one chromosome was observed.
5. New cytotypes was derived from interspecific hybrids involving *P. glaucum* and *P. orientale* containing $2n=37$ representing 28 glaucum and 9 orientale chromosomes. This represents fourth generation of recurrent addition of *P. glaucum* chromosomes into *P. orientale* background utilizing unreduced female gametes. These hybrids offer resource to study characters such as perenniality, apomixis and genome analysis.
6. Trispecific hybrids between *P. glaucum*, *P. orientale* and *P. squamulatum* was produced for the **first** time involving [F_1 (pearl millet x *P. squamulatum*) x BC_1 (*P. glaucum* x *P. orientale*)] cross, that contained $2n=44$ chromosomes representing 21 glaucum + 14 squamulatum + 9 orientale chromosomes. These hybrids are named as GOS hybrids. These hybrids offer material for production of multiple alien addition lines, apomixis genetics and genome analysis.

FIGURES

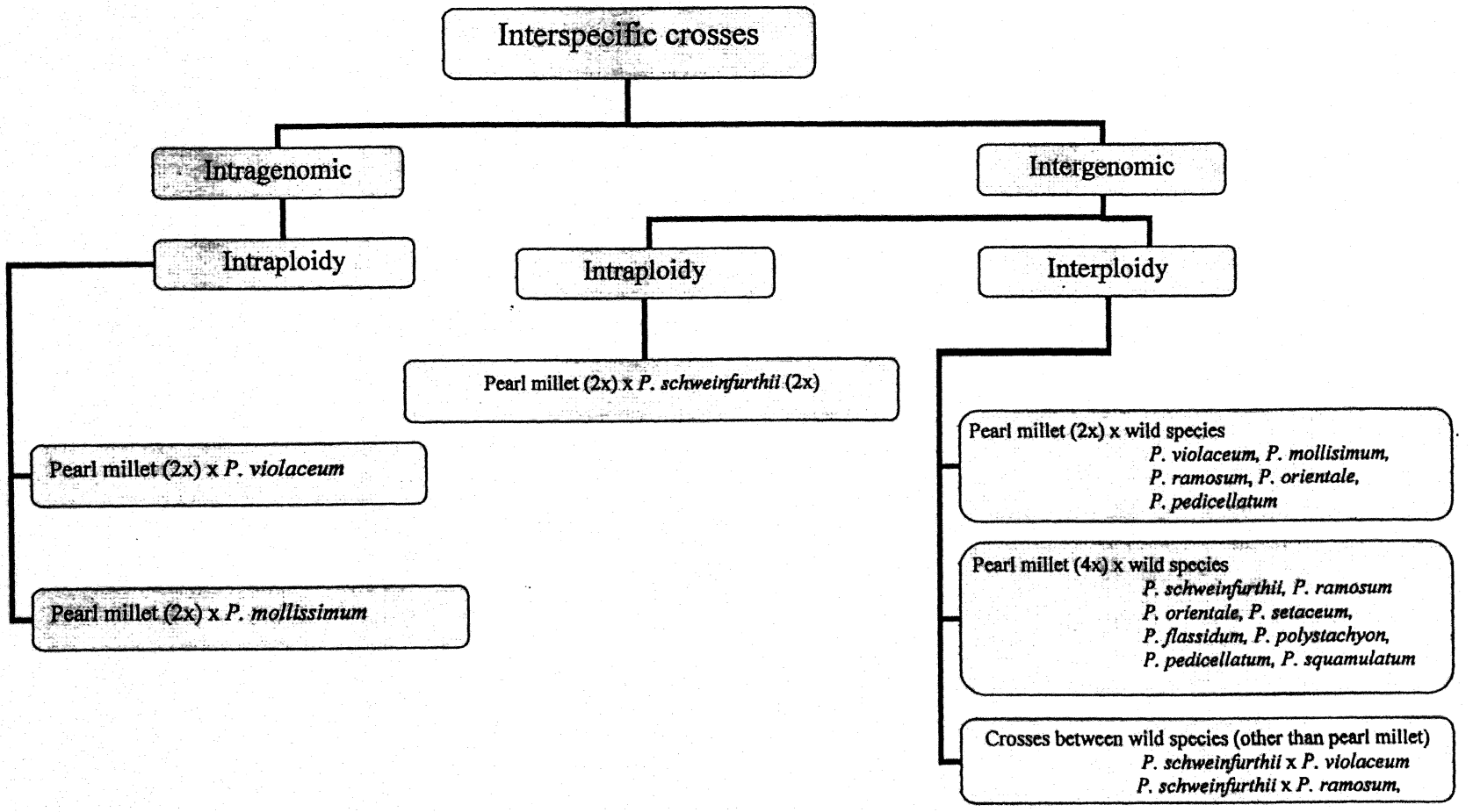
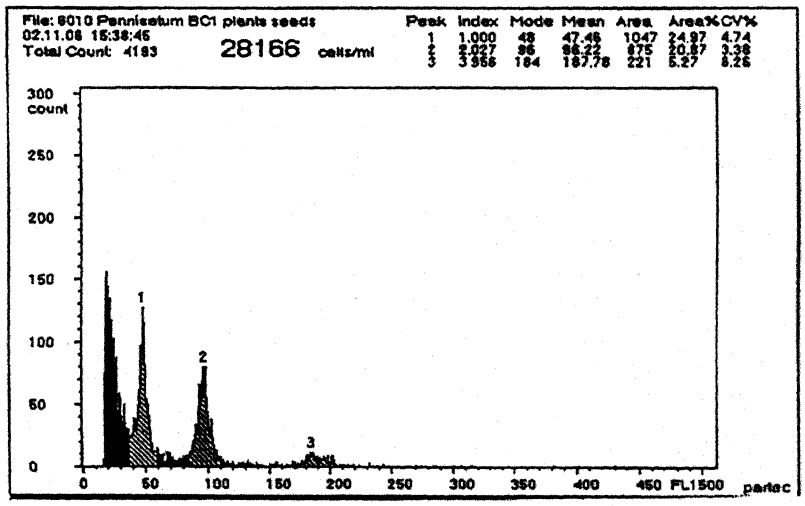


Figure 1. Synopsis of the work plan for the present study.

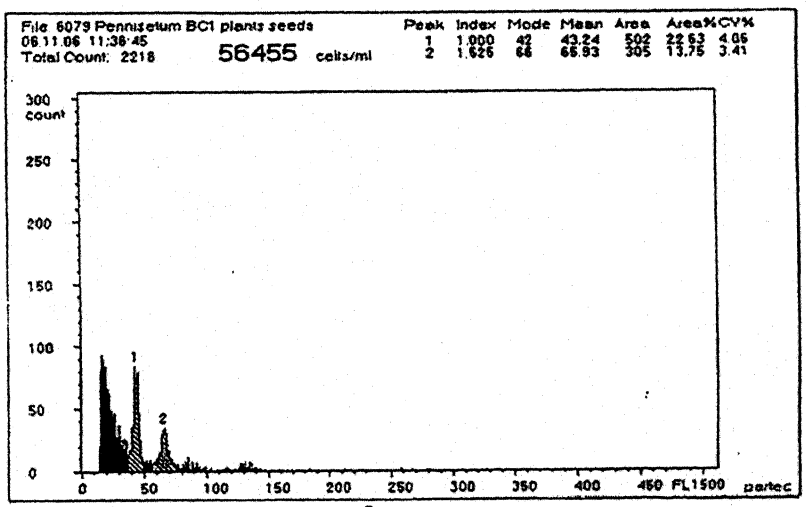
Figure 2: Flow cytometry of leaf samples of diploid and induced tetraploid pearl millet.

1. Peak 1; *P. glaucum* (Diploid 81A1), Peak 2; *P. glaucum* (tetraploid T1). Ratio of peaks **1:2.**
2. Peak 1; Control (*Panicum maximum*), Peak 2; *P. glaucum* (81A1 diploid). Ratio of peaks **1:1.5.**
3. Peak 1; Control (*Panicum maximum*), Peak 2; *P. glaucum* (induced tetraploid of 81A1). Ratio of peaks **1:3.**

FIG 2: FLOW CYTOMETRY OF LEAF SAMPLES OF DIPLOID AND INDUCED TETRAPLOID PEARL MILLET



1



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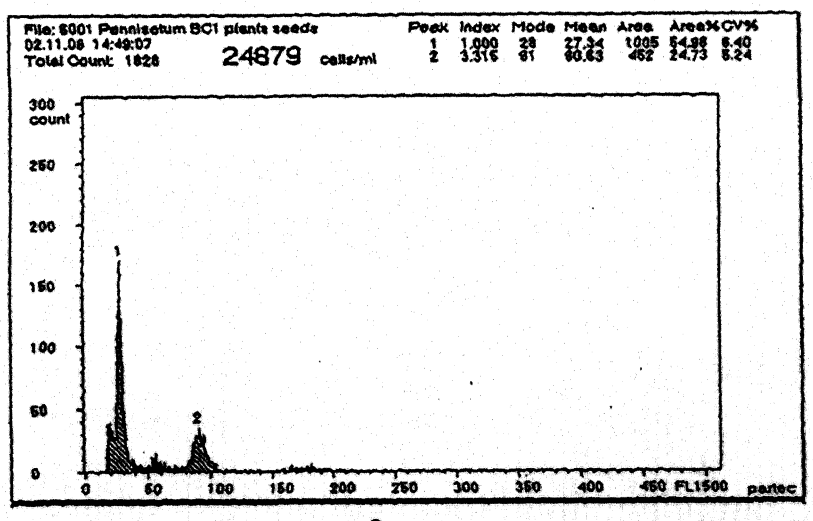
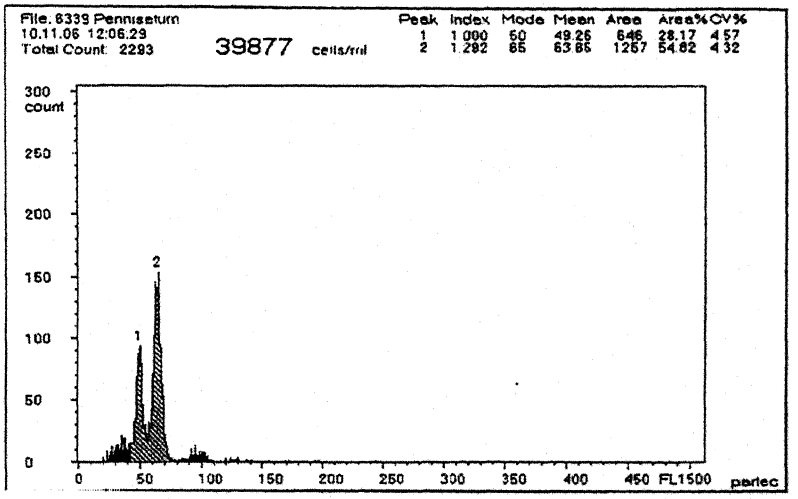


3

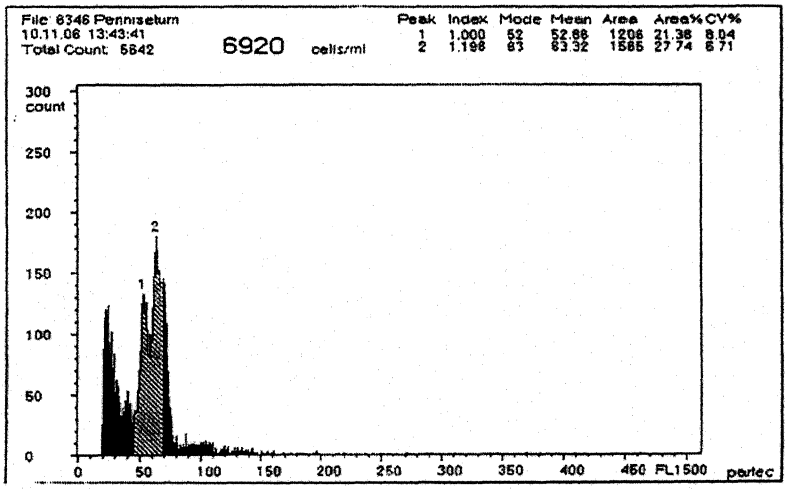
Figure 3: Characterization of pearl millet (4x), *P. squamulatum* and their hybrids (F_1 and BC_1) utilizing leaf flow cytometry.

1. Peak 1; *P. glaucum* (tetraploid T1), Peak 2; *P. squamulatum*. Ratio of peaks **1:1.292.**
2. Peak 1; F_1 Hybrid (*P. squamulatum* + *P. glaucum* (T1), Peak 2; *P. squamulatum.* Ratio of peaks **1:1.198.**
3. Peak 1; BC_1 (F_1 Hybrid + *P. glaucum* (T1), Peak 2; *P. squamulatum.* Ratio of peaks **1:1.190.**

FIG 3: CHARACTERIZATION OF PEARL MILLET (4x), *P. SQUAMULATUM* AND THEIR HYBRIDS (F_1 AND BC_1) UTILIZING LEAF FLOW CYTOMETRY



1



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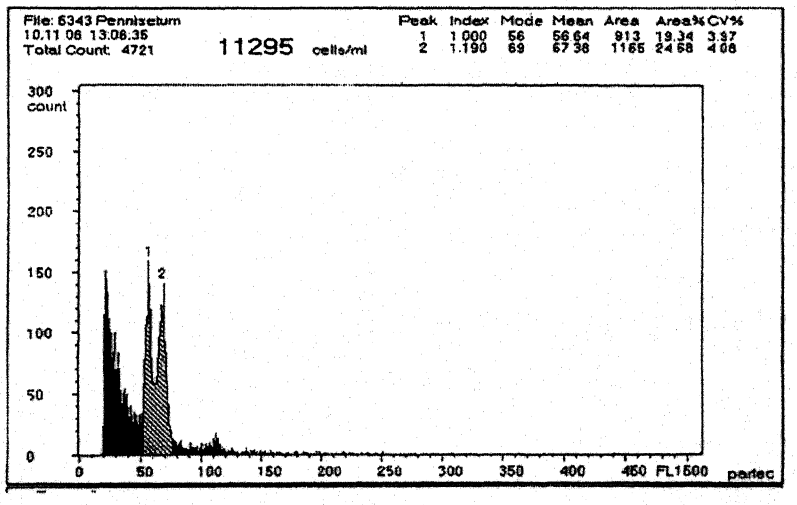

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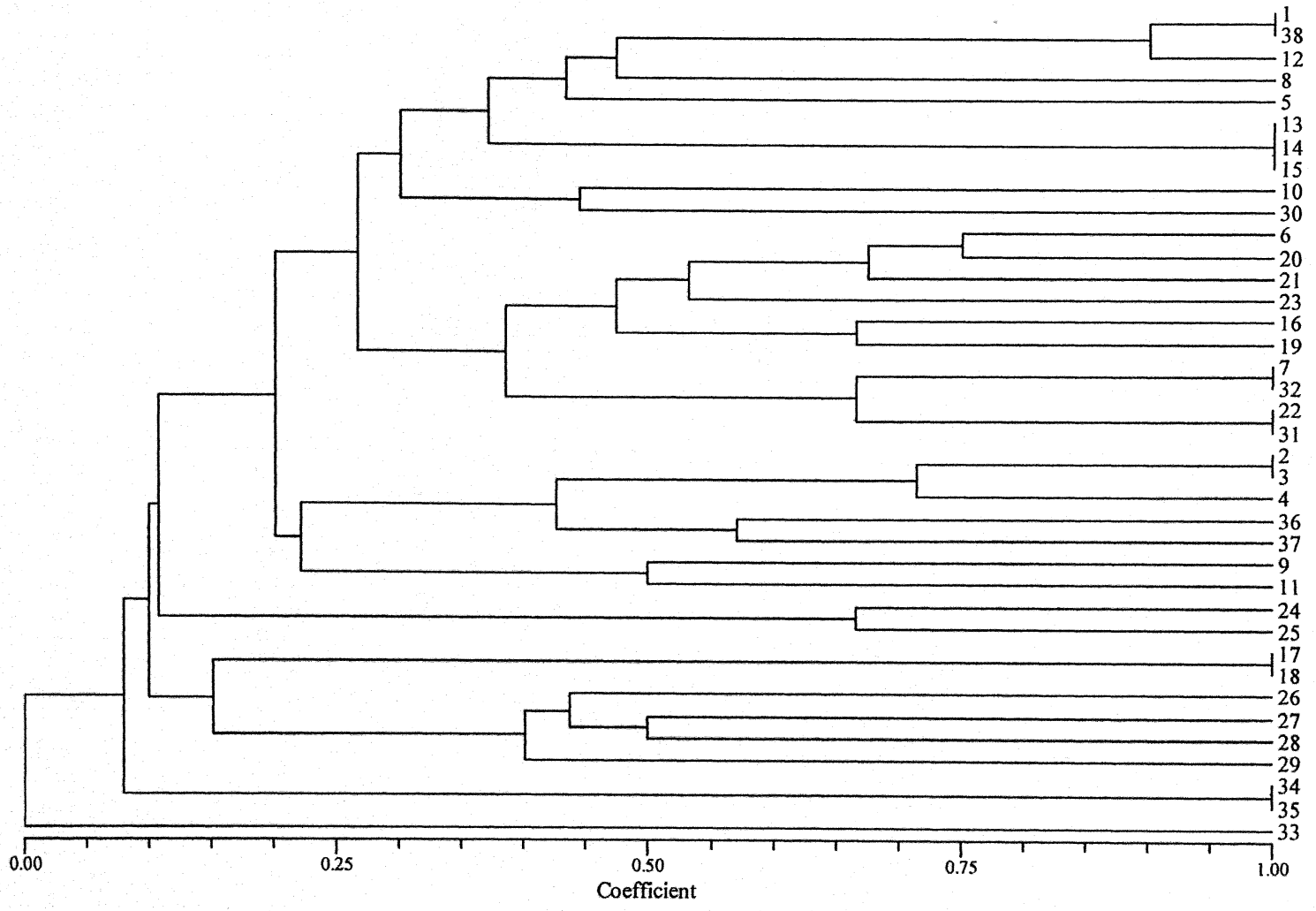


Figure 4: Dendogram based on Esterase isozyme banding pattern showing similarity among *Pennisetum* species.
* For sample numbers, refer Table 4.11

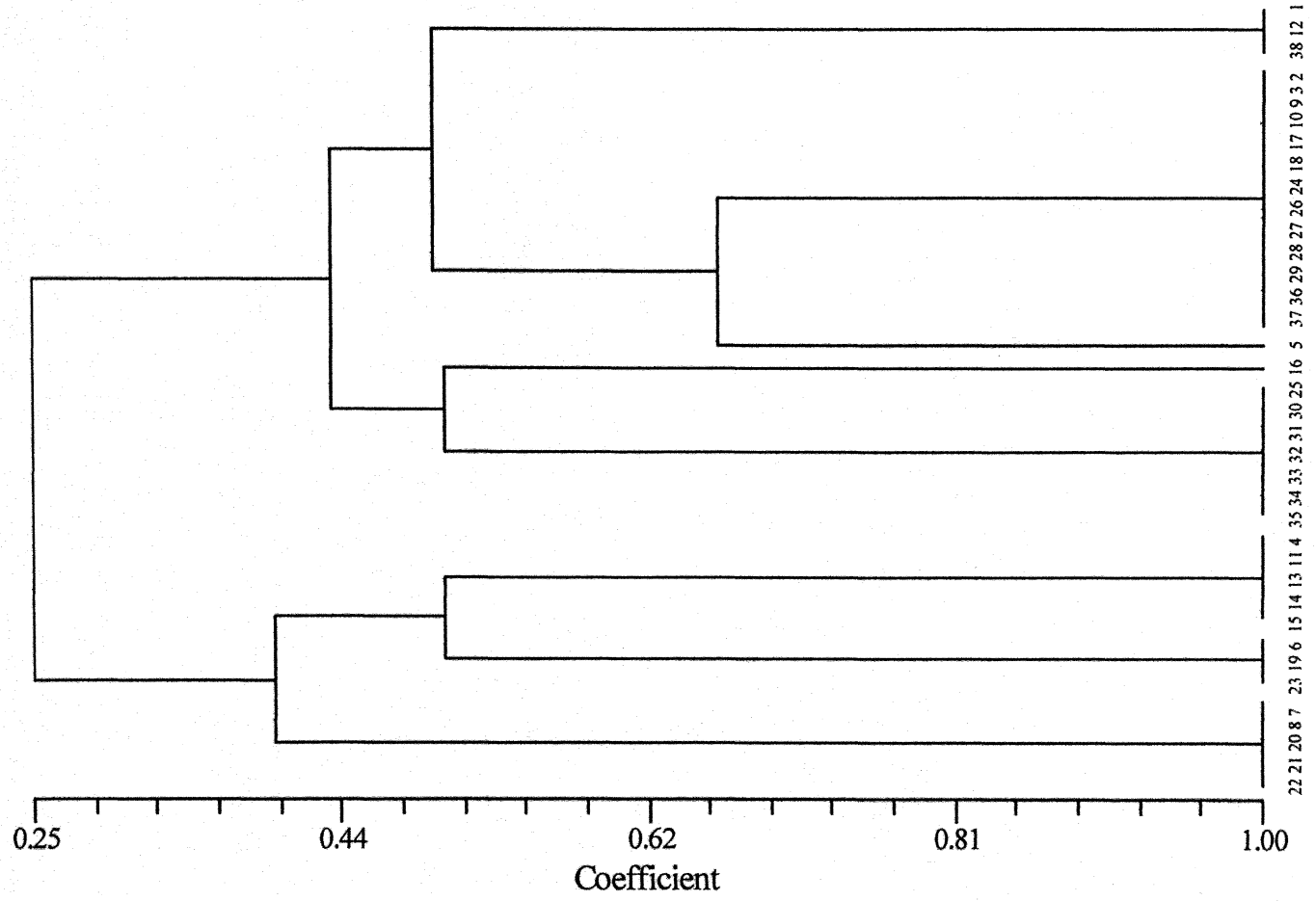


Figure 5: Dendogram based on Superoxide desmutase isozyme banding pattern showing similarity among *Pennisetum* species.
* For sample numbers, refer Table 4.12

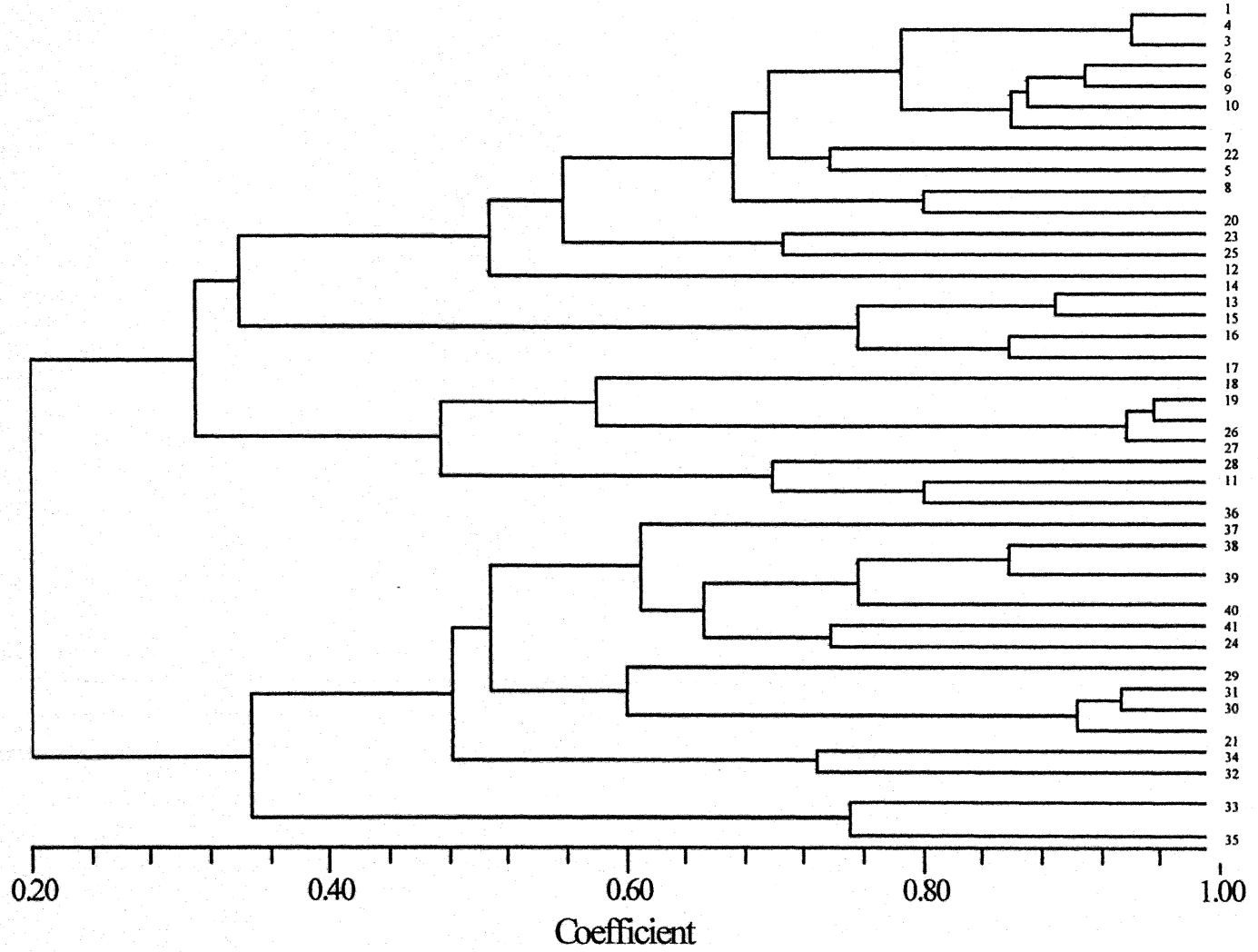


Figure 6: Dendogram based on Peroxidase isozyme banding pattern showing similarity among *Pennisetum* species.
* For sample numbers, refer Table 4.13.

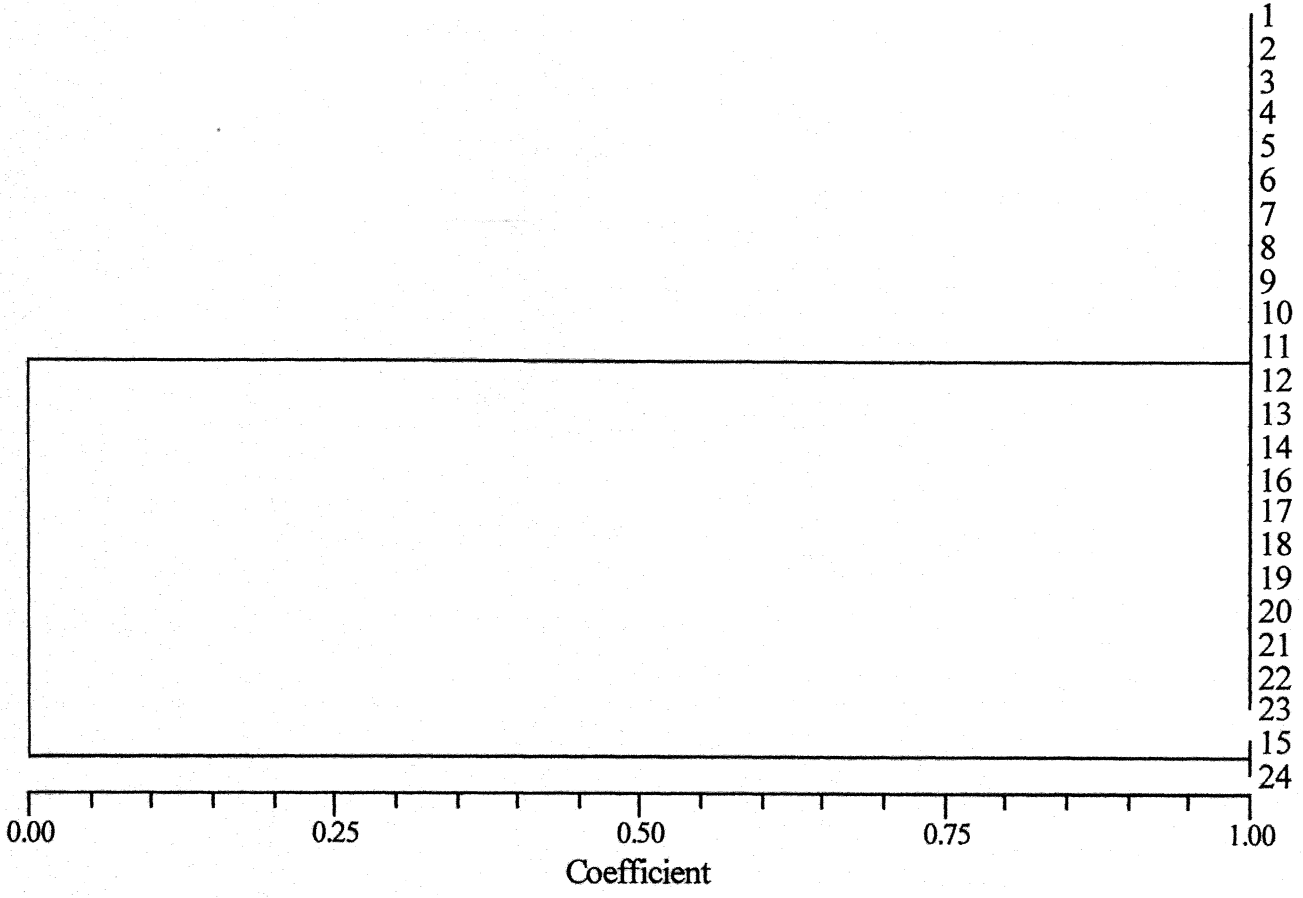


Figure 7: Dendogram based on Glutamate dehydrogenase isozyme banding pattern showing similarity among *Pennisetum* species.
* For sample numbers, refer Table 4.14.

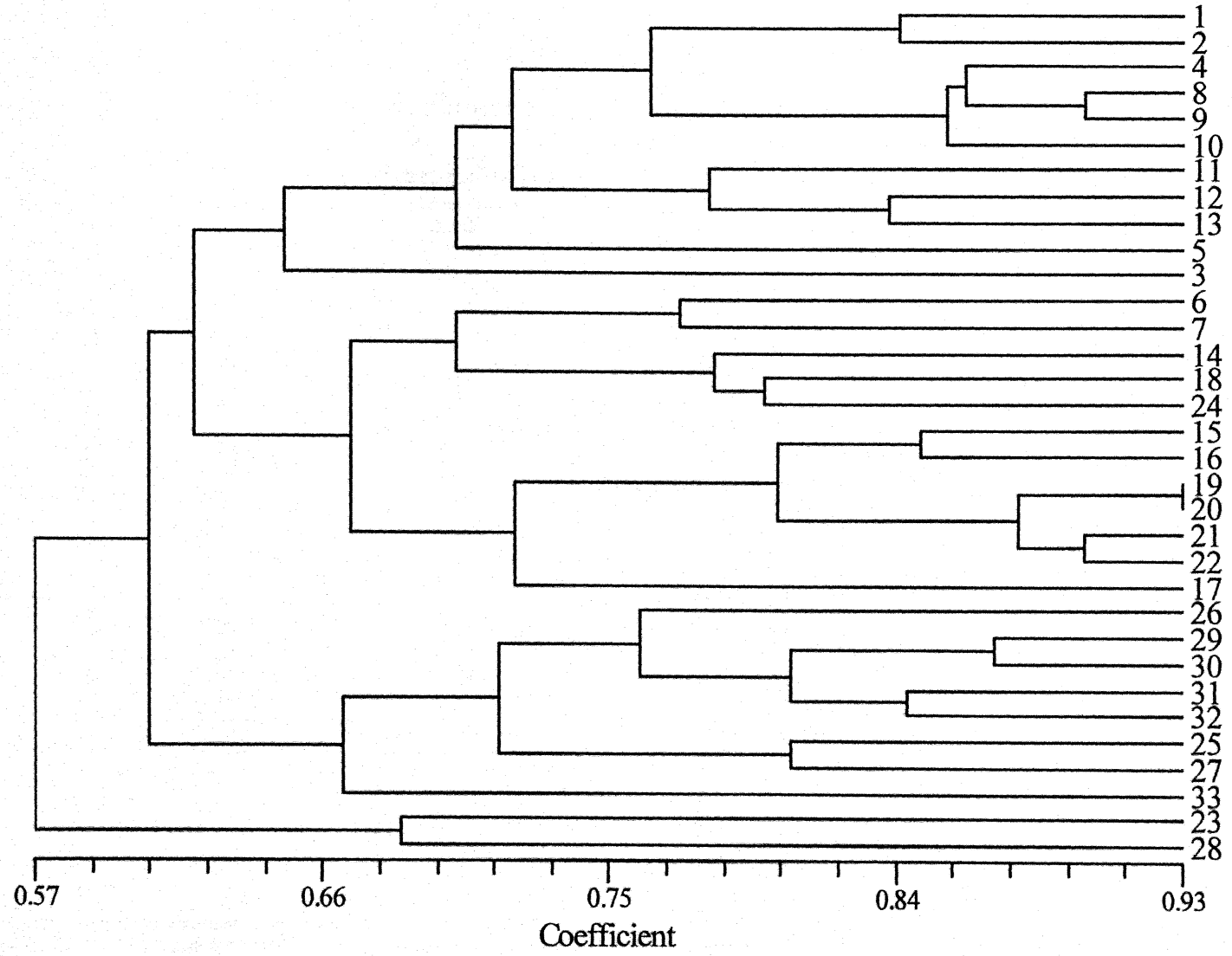
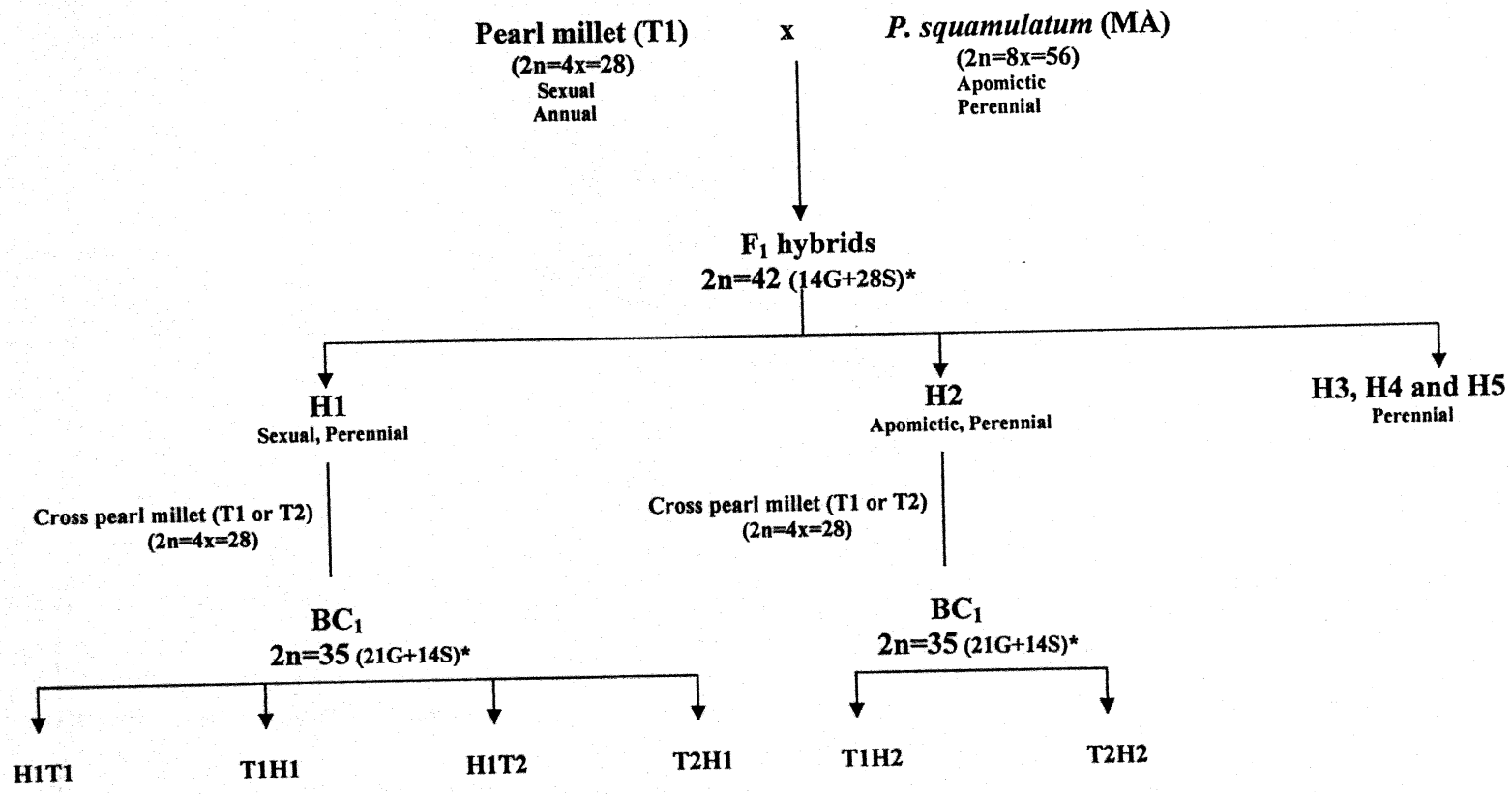


Figure 8: Dendogram based on protein and isozyme banding pattern in interspecific hybrids (F_1 and BC_1) and parents (pearl millet and *P. squamulatum*).

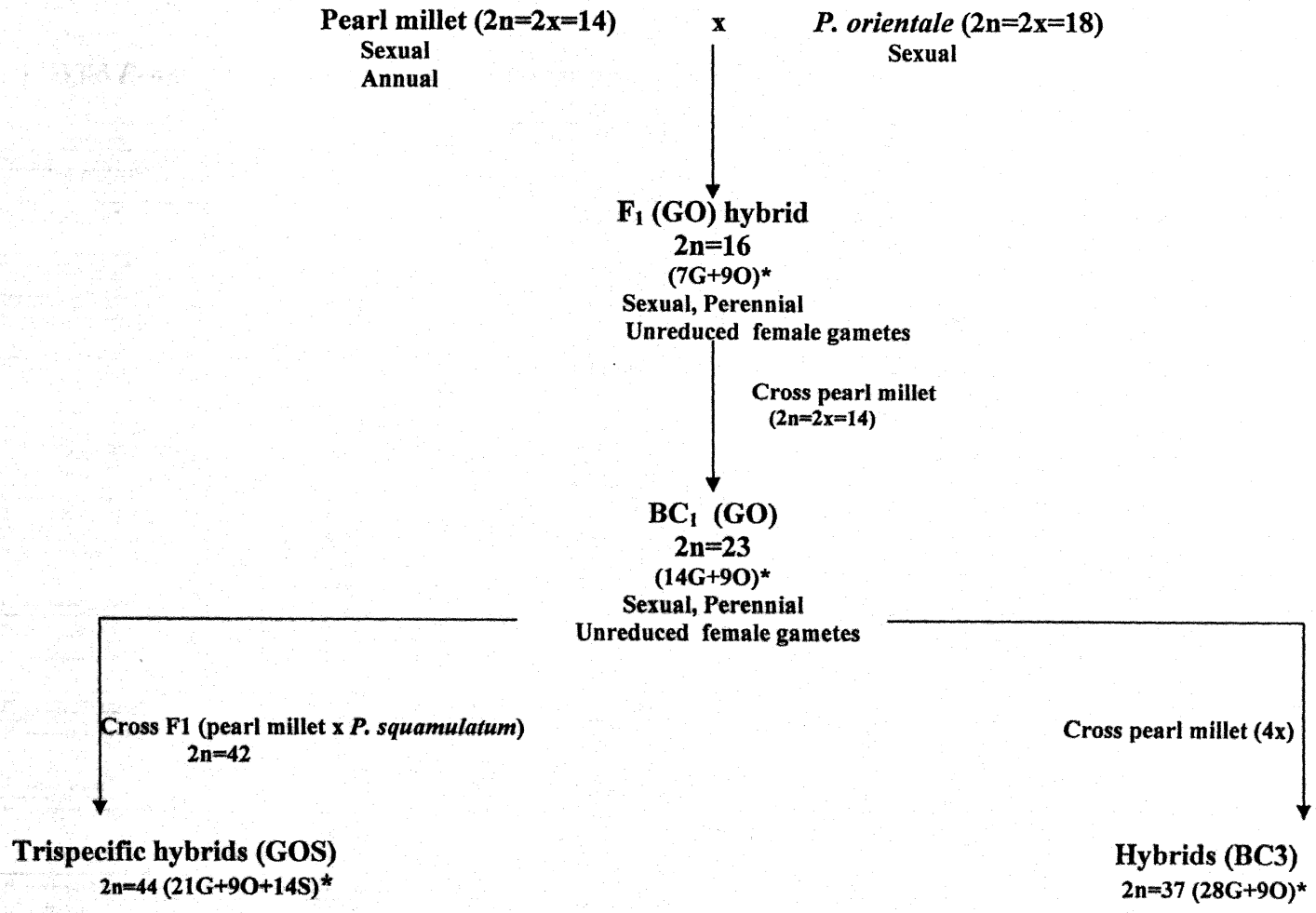
* For sample numbers, refer Table 4.39 to Table 4.42.

Figure 9: *P. glaucum* x *P. squamulatum*: F_1 and BC_1.



*G=glaucum chromosomes, S=squamulatum chromosomes
T1= *P. glaucum* IG 99-748, T2= *P. glaucum* IG 2000-01, MA= *P. squamulatum* IG 98-360, H1 and H2= F_1 hybrids

Figure 10: *P. glaucum* x *P. orientale*: F_1, BC_1 and new GO and GOS hybrids.



*G=glaucum chromosomes, O= orientale chromosomes, S=squamulatum chromosomes.
GO= hybrids between *P. glaucum* and *P. orientale*, GOS= trispecific hybrids between *P. glaucum*, *P. orientale* and *P. squamulatum*.

TABLES

Table 1.1. Wild *Pennisetum* species with potential for pearl millet improvement.

S.no	Species	x	2n	Reproduction	Lifecycle	Desirable characters	References
	Primary gene pool						
1	*P. glaucum**	7	14	Sexual	Annual	High food and fodder values	
2	*P. violaceum**	7	14	Sexual	Annual	Cytoplasmic male sterility	Burton and Athwal (1967), Marchais and Pernes (1985), Hanna (1989).
3	*P. mollisimum**	7	14	Sexual	Annual		
	Secondary gene pool						
4	*P. schweinfurthii**	7	14	Sexual	Annual	Rust resistance bigger grain size	
5	*P. purpureum**	7	28	Sexual	Perennial	Earliness, long inflorescence, leaf size, and male fertility restoration.	Burton (1944), Dujardin and Hanna (1989a).
	Tertiary gene pool						
6	*P. ramosum**	5	10	Sexual	Perennial	apomixis	Narayan (1955).
7	*P. mezianum*	8	32	Apomictic			Dujardin and Hanna (1984).
8	*P. dubium*	9	polyploids	Facultative			Gildenhuys and Brix (1958).
9	*P. flassidum**	9	18, 36, 45	Sex/ Apo	Perennial		Mehra and Ramanandan (1973), Chatterjee and Timothy (1969a), Mehra *et al.* (1968).
10	*P. hoheneckeri*	9	18, 19	Sex/ Apo	Perennial	apomixis	Narayan (1955), Parihar and Tripathi (1986)
11	*P. macrorum*	9	36, 54	Apomictic			
12	*P. orientale**	9	18, 36	Sex/ Apo	Perennial	Perenniality	Chatterjee and Timothy (1969b), Rangaswamy (1972), Jauhar (1981b)
13	*P. pedicellatum**	9	36, 54	Apomictic	Annual	Fodder values	Chatterjee and Pillai (1970), Jauhar (1981b).
14	*P. polystachyon**	9	54	Apomictic	Perennial	Disease resistance	Wilson and Hanna 1992, Birari (1981), Dujardin and Hanna (1984), Jauhar (1981b).
15	*P. setaceum**	9	27	Apomictic	Perennial	apomixis	Jauhar (1981b), Rangaswamy (1972).
16	*P. squamulatum**	9	56, 54	Apomictic	Perennial	Perenniality, apomixis, tolerance to abiotic and biotic stress	Dujardin and Hanna (1984), Kaushal *et al.* (2007).
17	*P. setosum*	9	54, 53, 56, 78	Apomictic		Fodder values, pereniality	Jauhar (1981b)
18	*P. villosum*	9	45	Apomictic	Perennial		Narayan (1955), Jauhar (1981b)

* Attempted for gene transfer as reported in literature.

Table 2.1: *Pennisetum* species belonging to different sections.

S.no.	Sections	Species
1	Penicillaria	*P. glaucum, P. violaceum, P. mollisimum, P. purpureum*
2	Brevivalvula	*P. polystachyon, P. pedicellatum*
3	Gymnothrix	*P. ramosum, P. mezianum, P. hoheneckeri, P. alopecuroides*
4	Heterostachya	*P. schweinfurthii, P. squamulatum, P. orientale*
5	Eu-Pennisetum	*P. villosum, P. setaceum*

Table 3.1: *Pennisetum* species used in the study.

S.no	Species	Total no. of accessions	Accessions no.	Source
1	*P. glaucum*	7	81B, 81A1, 81A4, 81A5, 81Av, IG 99-748 (T1), IG 2000-01 (T2)	IGFRI, ICRISAT, USDA
2	*P. violaceum*	8	PV 2433, IP 21532, IP 21586, IP 21579, IP 21634, IP 21534, IP 21632, IP 21524	IGFRI, ICRISAT
3	*P. mollisimum*	1	IP 21782	ICRISAT
4	*P. schweinfurthii*	6	PS 237, PS 2116, PS 233, IP 21929, IP 21931, IP 24214	IGFRI, ICRISAT
5	*P. ramosum*	3	IP 22180, IP 22137, IP 21935	ICRISAT
6	*P. orientale*	3	IP 21951, IP 22186, IG 04-165	ICRISAT IGFRI
7	*P. divisum*	2	IP 21962, IP 21957	ICRISAT
8	*P. setaceum*	1	IP 21949	ICRISAT
9	*P. setosum*	1	IP 21942	ICRISAT
10	*P. flassidum*	3	IP 22195, IP 22200, IP 22188	ICRISAT
11	*P. hoheneckeri*	3	IP 21954, IP 21953, IP 21952	ICRISAT
12	*P. polystachyon*	6	IP 22102, IP 22109, IP 22121, IP 21904, IP 21900, IP 21894	ICRISAT IGFRI
13	*P. pedicellatum*	8	IP 21971, IP 21790, IP 21879, IP 22095, IP 21890, IP 21883, NATP D-1, Agros 4	ICRISAT IGFRI
14	*P. villosum*	1	IP 21945	ICRISAT
15	*P. squamulatum*	3	IG 98-360 (or MA), IG 98-361 (or MB) IG 2000-36 (or MC)	IGFRI USDA

Table 3.2: *Pennisetum* species used for induction of polyploidy.

S. no.	Species	Accession no.	Chromosome no. 2n
	Primary gene pool		
1	*P. glaucum*	81A1, 81A4, 81A5	14
2	*P. glaucum*	IG 99-748 (or T1)	28
3	*P. violaceum*	IP 21586, IP 21579, IP 21532, IP 21641, IP 21634, IP 21632, IP 21634	14
	Secondary gene pool		
4	*P. schweinfurthii*	PS 233	14
	Tertiary gene pool		
5	*P. ramosum*	IP 22137, IP 21935, IP 22180	10

Table 3.3: Scheme of crossing.

S.no.	Female parent	Male parent
1	*P. glaucum* diploid MS* lines	*P. glaucum* (81B maintainer) *P. violaceum,* *P. mollisimum* *P. schweinfurthii* *P. ramosum* *P. orientale* *P. pedicellatum* *P. glaucum* x *P. squamulatum* (F_1 and BC_1) *P. glaucum* x *P. orientale* (F_1 and BC_1)
2	*P. glaucum* Tetraploid lines	*P. schweinfurthii* *P. ramosum* *P. orientale* *P. setaceum* *P. flassidum* *P. polystachyon* *P. pedicellatum* *P. squamulatum* *P. glaucum* x *P. squamulatum* (F_1 and BC_1) *P. glaucum* x *P. orientale* (F_1 and BC_1)
3	*P. glaucum* x *P. squamulatum* F_1 and BC_1	*P. glaucum* (diploid MS lines) *P. glaucum* (tetraploid lines)
4	*P. glaucum* x *P. orientale* F_1 and BC_1	*P. glaucum* (diploid MS lines) *P. glaucum* (tetraploid lines)

*MS= male sterile lines.

Table 3.4: Composition of L2, MS and RL basal media used in the present study.

S.no.	Components	L2 basal	MS basal	RL basal
1	KNO_3	20.8 x 10^3 µM	18.8 x 10^3 µM	10.4 x 10^3 µM
2	$NH4NO_3$	12.5 x 10^3 µM	20.6 x 10^3 µM	6.25 x 10^3 µM
3	KH_2PO_4	2.34 x 10^3 µM	1.25 x 10^3 µM	2.34 x 10^3 µM
4	$MgSO_4.7H_2O$	1.8 x 10^3 µM	1.5 x 10^3 µM	0.9 x 10^3 µM
5	$CaCl_2.H_2O$	4.1 x 10^3 µM	3.0 x 10^3 µM	2.0 x 10^3 µM
6	NaH_2PO_4	0.6 x 10^3 µM	-	0.3 x 10^3 µM
7	$FeSO_4.EDTA.7H_2O$	90 µM	100 µM	90 µM
8	$Na_2EDTA.2H_2O$	-	100 µM	-
9	$MnSO_4.4H_2O$	90 µM	100 µM	45 µM
10	H_3BO_3	82 µM	100 µM	41 µM
11	$ZnSO_4.7H_2O$	18 µM	30 µM	9 µM
12	KI	6 µM	5.0 µM	3 µM
13	$Na_2MoO_4.2H_2O$	1.7 µM	1.03 µM	0.85 µM
14	$CoCl_2.6H_2O$	0.42 µM	0.105 µM	0.21 µM
15	$CuSO_4.5H_2O$	0.4 µM	0.1 µM	0.2 µM
16	Myo-inositol	1.4 µM	550 µM	0.7 x 10^3 µM
17	Thiamine HCl	6 µM	2.96 µM	3.0 µM
18	Pyridoxine HCl	2.4 µM	24 x 10^3 µM	1.2 µM
19	Nicotinic acid	-	40.6 x 10^3 µM	8.5 µM
20	3- Aminopyridine			24 µM
21	Sucrose	73 x 10^3 µM	87.6 x 10^3 µM	44 µM

Table 3.5: Supplements used in various media.

S.No.	Components	L2 basal	MS basal	RL basal
1.	Agar	0.8%	0.7%	0.65%
2.	Auxin (NAA)	0.0008 mg/l	-	-
3.	Auxin (IAA)	-	-	0.21 mg/l
4.	Cytokinin (BAP)	0.150 mg/l	-	-

Table 4.1: Quantitative characters in *Pennisetum* species.

S.No.	Species	Accession no.	No. of tillers	Height of main tiller (cm)	No. of leaves/ tiller	Flag leaf		3rd leaf		Stem girth (cm)	No. of nodes/ tiller	Internode Length (cm)	Peduncle length (cm)	Spike		No. of florets/ spikelet
						length (cm)	width (cm)	length (cm)	width (cm)					length (cm)	width (cm)	
1	*P. glaucum*	81A1	7.0	105.0	10.7	24.0	2.5	39.0	2.8	1.0	10.0	12.3	27.3	25.7	2.7	4
2	*P. glaucum*	81B	4.7	92.3	6.3	42.7	2.5	44.7	2.7	1.0	4.7	11.0	28.0	22.3	3.3	4
3	*P. glaucum*	IG 99-748	3.3	136.7	6.7	11.7	3.5	71.7	3.7	1.7	6.0	15.3	38.3	25.7	1.1	4
4	*P. glaucum*	IG 2000-01	1.3	140.0	8.3	26.3	2.9	48.3	4.0	1.3	5.3	16.7	25.0	23.3	3.0	4
5	*P. violaceum*	IP 21634	7.7	148.3	9.0	10.7	0.7	22.7	1.0	0.8	7.3	20.3	13.5	9.0	1.3	4
6	*P. violaceum*	IP 21532	15.0	155.0	10.3	16.3	1.0	25.0	1.2	0.7	8.7	19.3	16.2	11.3	1.3	2
7	*P. violaceum*	IP 21534	2.7	220.0	10.0	25.0	2.1	38.3	2.3	0.7	9.7	22.0	22.0	14.7	1.6	4
8	*P. mollisimum*	IP 21782	8.7	280.0	13.3	24.0	1.0	45.0	1.6	0.7	11.7	21.8	18.7	14.7	1.8	4
9	*P. schweinfurthii*	IP 21929	12.0	200.0	10.0	23.0	2.0	42.0	2.8	0.8	8.0	20.0	38.0	19.0	2.5	10
10	*P. schweinfurthii*	IP 24214	10.3	158.3	8.7	19.0	1.7	48.3	2.2	0.8	6.3	23.2	23.8	17.5	3.3	2
11	*P. schweinfurthii* (4x)	PS 233	4.3	226.7	12.0	8.7	1.2	43.3	3.0	1.1	9.7	15.0	28.3	31.7	2.3	4
12	*P. ramosum*	IP 22137	8.9	195.0	10.2	16.9	1.6	44.6	2.7	0.9	8.0	19.4	30.1	22.7	2.7	4
13	*P. ramosum*	IP 21935	22.0	106.7	6.7	7.0	0.3	10.5	0.6	0.8	5.0	23.3	13.4	7.5	1.2	4
14	*P. orientale*	IP 21951	15.0	61.0	14.7	21.7	0.4	28.7	0.4	0.3	9.7	5.0	21.7	13.7	1.2	2
15	*P. orientale*	IG 04-165	28.0	90.0	8.0	13.0	0.6	31.0	1.0	0.4	8.0	9.0	21.0	12.0	0.3	4
16	*P. divisum*	IP 21957	31.0	77.3	9.7	9.8	0.4	18.2	0.5	0.3	9.3	8.0	15.8	8.0	0.7	2
17	*P. divisum*	IP 21962	35.3	85.1	8.2	12.3	0.4	23.7	0.6	0.3	8.1	6.7	16.1	9.0	1.1	5
18	*P. setaceum*	IP 21949	110.7	123.3	5.7	26.7	0.3	59.0	0.6	0.5	4.3	16.7	46.0	26.0	2.3	4
19	*P. flassidum*	IP 22195	19.3	105.7	8.0	22.3	0.5	38.3	0.6	0.3	8.0	9.8	23.0	16.3	0.7	2
20	*P. flassidum*	IP 22200	45.7	105.3	9.3	24.0	0.6	38.0	0.7	0.3	7.3	9.2	21.3	15.2	0.7	4
21	*P. hoheneckeri*	IP 21954	35.0	50.0	6.0	22.7	0.3	35.0	0.4	0.3	4.7	10.7	24.7	11.7	0.7	1
22	*P.setosum*	IP 21942	106.0	166.0	18.0	20.0	1.5	40.0	1.5	1.0	1.0	10.0	25.0	15.0	1.0	4
23	*P. polystachyon*	IP 22102	18.0	166.7	8.7	8.2	0.6	15.5	1.1	0.5	8.7	19.5	17.7	13.7	1.5	2
24	*P. polystachyon*	IP 22109	17.0	150.0	7.3	6.8	0.4	14.3	0.6	0.4	7.3	19.2	23.3	17.7	1.3	1
25	*P. polystachyon*	IP 22121	23.7	163.3	9.7	5.3	0.3	13.5	0.7	0.4	9.7	17.5	24.0	19.0	0.9	2
26	*P. pedicellatum*(6x)	NATP D-1	12.7	115.0	7.0	4.3	1.2	17.3	1.1	0.4	6.3	17.0	18.7	12.3	1.3	3
27	*P. pedicellatum*(6x)	IP 21971	16.3	152.7	8.3	7.3	0.5	22.5	1.3	0.7	8.3	12.7	11.7	12.5	1.4	3
28	*P. pedicellatum*(4x)	IP 22095	30.0	120.3	7.0	13.3	0.4	20.3	0.9	0.3	7.0	17.0	15.8	14.5	1.5	6
29	*P. pedicellatum*(8x)	Agros 4	41.7	127.0	5.7	12.3	0.6	21.7	1.0	0.4	5.7	12.0	15.7	12.7	1.3	4
30	*P. villosum*	IP 21945	32.0	54.0	6.0	14.0	0.2	24.0	0.4	0.2	6.0	9.0	19.0	10.0	3.0	2
31	*P. squamulatum*	IG 98-360	20.0	186.3	10.7	31.3	1.4	68.7	1.6	0.6	10.7	16.0	57.0	31.7	2.0	14
32	*P. squamulatum*	IG 2000-36	106.7	270.0	10.3	41.7	1.5	56.3	1.6	0.6	7.7	29.0	45.7	21.0	1.0	5

Table 4.2: Grouping of various accessions of *Pennisetum* species in different clusters.

Cluster no.	Plant no.	Accessions
1	2,3,4	*P. glaucum* (81B, IG 99-748 or T1, IG 2000-01 or T2)
2	5,6,13,23,24,25,26,27,28,29	*P. violaceum* (IP 21634, IP 21532), *P. ramosum* (IP 21935), *P. polystachyon* (IP 22102, IP 22109, IP 22121), *P. pedicellatum* (NATP D-1 (6x), IP 21971(6x), IP 22095(4x), Agros 4(8x))
3	18,31,32	*P. setaceum* (IP 21949), *P. squamulatum* (IG 98-360, IG 2000-36)
4	12	*P. ramosum* (IP 22137)
5	1,7,8,9,10,11	*P. glaucum* (81A1), *P. violaceum* (IP 21534), *P. mollisimum* (IP 21782), *P. schweinfurthii* (IP 21929, IP 24214, PS 233)
6	14,15,16,17,19,20,21,22,30	*P. orientale* (IP 21951, IG 04-165), *P. divisum* (IP 21957, IP 21962), *P. flassidum* (IP 22195, IP 22200), *P. hoheneckeri* (IP 21954), *P. setosum* (IP 21942), *P. villosum* (IP 21945)
Total	32	

Table 4.3: Distance between cluster centroids.

Cluster no.	1	2	3	4	5	6
1	0.000					
2	5.900	0.000				
3	5.851	5.780	0.000			
4	7.642	5.333	7.449	0.00		
5	3.813	3.915	4.622	6.632	0.00	
6	6.182	2.343	5.707	5.212	4.737	0.00

Table 4.4: Morphological characters (mean± standard deviation) in different clusters of species.

Characters → Cluster no.↓	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	3.11± 1.68	123.00± 26.61	7.11± 1.07	26.89± 15.51	2.98± 0.50	54.89± 14.65	3.46± 0.68	1.33± 0.33	5.33± 0.67	14.33± 2.96	30.44± 6.99	23.78± 1.71	2.49± 1.19	4.00± 0.00
2	20.40± 9.68	140.50± 21.34	7.97± 1.47	9.17± 3.85	0.62± 0.30	18.33± 4.78	0.94± 0.25	0.53± 0.17	7.40± 1.46	17.78± 3.42	16.99± 4.08	13.02± 3.50	1.32± 0.16	2.57± 0.96
3	67.44± 45.48	193.22± 73.58	8.89± 2.80	33.22± 7.68	1.09± 0.66	61.33± 6.49	1.26± 0.57	0.53± 0.06	7.56± 3.17	20.56± 7.32	49.56± 6.45	26.22± 5.34	1.77± 0.68	7.33± 5.77
4	14.00± 0.00	86.67± 0.00	10.00± 0.00	78.33± 0.00	0.37± 0.00	12.50± 0.00	0.67± 0.00	0.50± 0.00	4.67± 0.00	21.17± 0.00	10.33± 0.00	6.33± 0.00	1.13± 0.00	4.00± 0.00
5	6.61± 2.79	198.33± 60.42	10.78± 1.66	20.33± 6.12	1.71± 0.55	42.11± 4.11	2.43± 0.49	0.85± 0.18	9.28± 1.79	18.67± 4.38	26.31± 6.59	21.03± 6.77	2.36± 0.64	4.22± 2.05
6	32.70± 10.02	84.30± 28.54	9.44± 3.55	17.61± 5.25	0.56± 0.37	30.54± 6.85	0.68± 0.38	0.37± 0.24	7.03± 1.91	8.52± 1.89	20.30± 3.38	12.69± 3.08	1.06± 0.64	2.63± 1.11

1=number of tillers, 2= height of main tiller, 3= number of leaves, 4= flag leaf length, 5= flag leaf width, 6= 3rd leaf length, 7= 3rd leaf width, 8= stem girth, 9= number of nodes, 10= internode length, 11= peduncle length, 12=spike length, 13= spike width, 14= number of florets / spikelet.

Table 4.5: Variability estimates for various metric traits in species.

Characters →	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Range	1.3-110.7	50.0-280.0	5.6-16.0	4.3-78.3	0.3-3.5	10.5-71.7	0.4-4.0	0.2-1.7	4.0-11.7	5.0-29.0	10.3-57.0	6.3-31.7	0.7-3.3	1.0-14.0
Mean	23.8	137.2	8.9	19.7	1.1	33.5	1.4	0.6	7.4	15.4	23.7	16.5	1.6	3.5
CV	34.9	17.9	14.9	20.4	40.3	13.6	17.8	20.0	23.5	20.9	19.2	15.3	20.4	27.5
GCV	91.6	40.4	26.6	72.1	77.7	47.7	70.4	54.4	23.9	35.2	43.7	39.8	47.7	64.5
PCV	98.0	44.2	30.5	74.9	87.6	49.6	72.6	57.9	33.6	41.0	47.7	42.7	51.9	70.1
GA	42.1	104.3	4.3	28.2	1.5	31.7	1.9	0.7	2.6	9.6	19.6	12.6	1.4	4.3
Heritability	0.9	0.8	0.8	0.9	0.8	0.9	0.9	0.9	0.5	0.7	0.8	0.9	0.8	0.8

1=number of tillers, 2= height of main tiller, 3= number of leaves, 4= flag leaf length, 5= flag leaf width, 6= 3rd leaf length, 7= 3rd leaf width, 8= stem girth, 9= number of nodes, 10= internode length, 11= peduncle length, 12=spike length, 13= spike width, 14= number of florets / spikelet.
CV=coefficient of variation, GCV= genotypic coefficient of variation, PCV= phenotypic coefficient of variation, GA= genetic advance values

Table 4.6: Genotypic correlation coefficient based on morphological observations in the *Pennisetum* species.

Characters →	1	2	3	4	5	6	7	8	9	10	11	12	13
1	1.000												
2	-0.119	1.000											
3	-0.218	0.400	1.000										
4	0.077	0.012	0.167	1.000									
5	-0.450	0.329	0.105	0.151	1.000								
6	0.148	0.380	0.111	0.260	0.673	1.000							
7	-0.492	0.428	0.138	0.102	0.974	0.606	1.000						
8	-0.445	0.354	0.175	0.043	0.888	0.529	0.914	1.000					
9	-0.422	0.560	0.507	-0.240	-0.015	0.058	0.055	-0.117	1.000				
10	-0.075	0.709	0.015	0.200	0.196	0.073	0.204	0.223	0.108	1.000			
11	0.322	0.372	0.079	0.218	0.447	0.855	0.374	0.275	0.132	0.160	1.000		
12	0.006	0.425	0.155	0.066	0.607	0.787	0.669	0.547	0.248	0.064	0.835	1.000	
13	-0.262	0.154	-0.062	0.157	0.503	0.402	0.610	0.455	-0.057	0.155	0.264	0.485	1.000
14	-0.026	0.227	0.113	0.295	0.356	0.541	0.286	0.195	0.158	0.143	0.633	0.485	0.395

1=number of tillers, 2= height of main tiller, 3= number of leaves, 4= flag leaf length, 5= flag leaf width, 6= 3rd leaf length, 7= 3rd leaf width, 8= stem girth, 9= number of nodes, 10= internode length, 11= peduncle length, 12=spike length, 13= spike width, 14= number of florets / spikelet.

Table 4.7: Phenotypic correlation coefficient based on morphological observations in the *Pennisetum* species.

Characters →	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	1.000													
2	-0.088	1.000												
3	-0.181	0.364	1.000											
4	0.078	0.004	0.125	1.000										
5	-0.354	0.250	0.098	0.193	1.000									
6	0.119	0.308	0.095	0.271	0.584	1.000								
7	-0.442	0.398	0.143	0.105	0.862	0.579	1.000							
8	-0.373	0.324	0.143	0.041	0.761	0.471	0.858	1.000						
9	-0.233	0.409	0.461	-0.167	-0.020	0.020	0.075	-0.068	1.000					
10	-0.067	0.627	-0.067	0.187	0.131	0.079	0.208	0.257	0.022	1.000				
11	0.272	0.349	0.048	0.209	0.403	0.755	0.367	0.258	0.046	0.165	1.000			
12	0.003	0.396	0.093	0.079	0.547	0.710	0.631	0.493	0.137	0.109	0.801	1.000		
13	-0.216	0.174	-0.056	0.152	0.485	0.318	0.564	0.414	-0.052	0.176	0.275	0.477	1.000	
14	-0.024	0.214	0.136	0.252	0.262	0.499	0.276	0.163	0.125	0.132	0.546	0.402	0.316	1.000

1=number of tillers, 2= height of main tiller, 3= number of leaves, 4= flag leaf length, 5= flag leaf width, 6= 3rd leaf length, 7= 3rd leaf width, 8= stem girth, 9= number of nodes, 10= internode length, 11= peduncle length, 12=spike length, 13= spike width, 14= number of florets / spikelet.

Table 4.8: Qualitative characters in *Pennisetum* species.

S.no	Species	Accession no.	Node		Leaf base		Stigma		Awn colour	Life cycle	Habit
			colour	hairiness	colour	hairiness	colour	bifid/ trifid			
1	*P. glaucum*	81A1	V	NH	LV	H	W	B	Y	A	E
2	*P. glaucum*	81B	V *	NH	Y	H	W	B	Y	A	E
3	*P. glaucum*	IG 99-748 (T1)	Y	H	Y	H	W	B	Y	A	E
4	*P. glaucum*	IG 2000-01 (T2)	LV	H	Y	H	W	B	Y	A	E
5	*P. violaceum*	IP 21634	V	H	V	H	W	B	Y	A	E
6	*P. violaceum*	IP 21532	Y	LH	Y	H	W	B	Y	A	E
7	*P. violaceum*	IP 21534	DV	H	DV	H	W	B	LV	A	E
8	*P. mollisimum*	IP 21782	DV	H	DV	H	W	B	V	A	E
9	*P. schweinfurthii*	IP 21929	G	NH	Y	NH	W	B	DV	A	E
10	*P. schweinfurthii*	IP 24214	G	NH	Y	H	W	B	Y	A	E
11	*P. schweinfurthii*	PS 233	G	NH	Y	H	W	B	DV	A	E
12	*P. ramosum*	IP 22137	G	NH	Y	NH	W	B	Y	B	D
13	*P. ramosum*	IP 21935	G	NH	Y	NH	W	B	Y	B	D
14	*P. orientale*	IP 21951	LV	LH	Y+V	LH	DV	B	V	P	E
15	*P. orientale*	IG 04-165	LV	NH	LV	NH	DV	B	V	P	E
16	*P. divisum*	IP 21957	Y+V	LH	Y	H	DV	B	DV	P	D
17	*P. divisum*	IP 21962	Y+V	NH	Y	LH	V	B	DV	P	D
18	*P. setaceum*	IP 21949	Y	NH	Y	NH	V	B	V	P	E
19	*P. flassidum*	IP 22195	Y	NH	LV	LH	DV	B	DV	P	D
20	*P. flassidum*	IP 22200	V *	NH	V	NH	DV	B	DV	P	D
21	*P. hoheneckeri*	IP 21954	Y	NH	Y	LH	W	B	Y	P	E
22	*P. setosum*	IP 21942	V	H	Y	H	W	B	Y	P	D
23	*P. polystachyon*	IP 22102	V	NH	LV	LH	V	B+T	DV	P	E
24	*P. polystachyon*	IP 22109	LV	NH	LV	H	LV	B	LV	P	E
25	*P. polystachyon*	IP 22121	DV	NH	LV	LH	V	B	LV	P	E
26	*P. pedicellatum*	NATP D-1	Y	NH	V	LH	W	B	V	A	E
27	*P. pedicellatum*	IP 21971	Y+V	NH	Y+V	H	V	B	V	A	E
28	*P. pedicellatum*	IP 22095	G	NH	Y	H	W	B	Y	A	E
29	*P. pedicellatum*	Agros 4	Y	NH	Y	H	W	B	Y	P	E
30	*P. villosum*	IP 21945	Y	NH	Y	LH	W	B	Y	P	D
31	*P. squamulatum*	IG 98-360 (MA)	Y	H	Y	H	DV	B+T	Y	P	E
32	*P. squamulatum*	IG 98-361 (MB)	Y	H	Y	LH	V	B+T	Y	P	E
33	*P. squamulatum*	IG 2000-36 (MC)	Y	H	Y	LH	V	B+T	Y	P	E

NH= non hairy, H= hairy, LH= less hairy, W= white, Y= yellow, G= green, LV= light violet, V= violet, DV= dark violet, V * =violet above node, Y+V= yellow and violet both colours observed in the same plant, B= bifid, B+T= bifid and trifid both stigma found in the same spike, A= annual, P=perennial, E= erect, D=decumbent.

Table 4.9: Cytological observations of *Pennisetum* species.

S.no.	Species	Accession no.	2n	Ploidy	PMC Studied	Average chromosomal association & range* at diakinesis							Chiasmata frequency/cell (%) & range*	Pollen stainability %
						I	II	III	IV	V	VI	VII		
1	*P. glaucum*	81B	14	2x	25		7						14.17	93
													(12-17)	
2	*P. glaucum*	81A1	14	2x	30		7						13.9	sterile
													(11-16)	
3	*P. glaucum*	81A4	14	2x	25		7						13.6	sterile
													(12-14)	
4	*P. glaucum*	IG 99-748 (or T1)	28	4x	25	1.2	7.16	0.96	2.4				19.64	84
						(0-3)	(3-12)	(0-4)	(0-5)				(17-24)	
5	*P. glaucum*	IG 2000-01 (or T2)	28	4x	20	0.35	10.15	0.25	1.65				22.4	65
						(0-2)	(6-14)	(0-2)	(0-3)				(19-25)	
6	*P. violaceum*	PV 2433	14	2x	20	0.1	6.95						12.95	98
						(0-2)	(6-7)						(10-14)	
7	*P. violaceum*	IP 21532	14	2x	20	0.1	6.95						12.75	98
						(0-2)	(6-7)						(11-14)	
8	*P. violaceum*	IP 21586	14	2x	15		7						13.87	98
													(13-14)	
9	*P. mollisimum*	IP 21782	14	2x	25	0.32	6.84						12.04	77
						(0-4)	(5-7)						(9-13)	
10	*P. schweinfurthii*	PS 233	14	2x	15	0.26	6.86						9.8	67
						(0-2)	(6-7)						(9-12)	
11	*P. ramosum*	IP 22180	10	2x	17	0.23	4.88						9.76	99
						(0-4)	(3-5)						(6-10)	
12	*P. ramosum*	IP 22137	10	2x	25		5						9.96	96
													(9-10)	
13	*P. orientale*	IG 04-165	54	6x	15	0.13	22.6		2.13				2.1	85
						(0-2)	(19-26)		(0-4)				(0-4)	
14	*P. divisum*	IP 21962	36	4x	25	0.52	15.8	0.6	0.52				31.84	86
						(0-4)	(8-18)	(0-3)	(0-3)				(24-36)	
15	*P. setaceum*	IP 21949	27	3x	25	7.16	9.72						18.68	24
						(3-15)	(6-12)						(12-22)	
16	*P. flassidum*	IP 22195	45	5x	25	3.96	17.48	1.28	0.56				31.32	47
						(1-10)	(12-22)	(0-3)	(0-2)				(24-41)	
17	*P. hoheneckeri*	IP 21954	18	2x	25	0.08	8.96						15.32	65
						(0-2)	(8-9)						(11-18)	
18	*P. polystachyon*	IP 22102	54	6x	20	1.8	24.05	0.3	0.8				ND	65
						(0-8)	(20-27)	(0-6)	(0-2)					
19	*P. pedicellatum*	NATP D-1	54	6x	25	2.2	11.48	1.88	4.36	0.04	0.88		ND	65
						(0-6)	(5-20)	(0-4)	(2-7)	(0-1)	(0-2)			
20	*P. pedicellatum*	IP 21971	54	6x	20	0.1	24.8	0.75	0.2	0.1	0.15		ND	63
						(0-1)	(18-27)	(0-4)	(0-2)	(0-1)	(0-3)			
21	*P. pedicellatum*	IP 21790	54	6x	20	0.85	13.75	0.35	4.2		1.3		43.15	87
						(0-4)	(10-20)	(0-2)	(2-7)		(0-3)		(37-51)	
22	*P. pedicellatum*	IP 21879	54	6x	20	1.05	20.05	0.75	2.65				44.45	73
						(0-4)	(10-25)	(0-3)	(1-7)				(35-52)	
23	*P. pedicellatum*	IP 22095	36	4x	25	0.08	17.6	0.24					ND	54
						(0-2)	(15-18)	(0-2)						
24	*P. pedicellatum*	Agros 4	72	8x	30	1.03	20.43	3.47	4.3	0.33	0.1	0.03	52.2	69
						(0-5)	(12-25)	(1-6)	(1-7)	(0-2)	(0-2)	(0-1)	(42-60)	
25	*P. squamulatum*	IG 98-360 (or MA)	56	8x	20		22.3	0.25	1.7	0.05	0.6		53.3	71
							(10-28)	(0-2)	(0-6)	(0-1)	(0-2)		(46-56)	
26	*P. squamulatum*	IG 98-361 (or MB)	56	8x	20	2.85	15.65	1.7	3.25	0.15	0.5		46.1	71
						(0-7)	(10-23)	(0-6)	(1-6)	(0-1)	(0-1)		(31-53)	
27	*P. squamulatum*	IG 2000-36 (or MC)	56	8x	20	0.3	19.8	1.5	2.9				44.4	74
						(0-2)	(16-26)	(0-4)	(1-6)				(39-51)	

*Figures in parentheses denote range. ND- not done (the chiasmata could not be recorded because of very small size of chromosomes).

Table 4.10: Rm values of different isozymes in *Pennisetum* species.

Enzyme	E.C. No.	Band numbers																									
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Esterase	3.1.1.2	0.03	0.08	0.13	0.16	0.18	0.19	0.22	0.24	0.29	0.32	0.35	0.36	0.41	0.42	0.43	0.44	0.46	0.57	0.6	0.61	0.62	0.66	0.7	0.72	0.74	0.76
SOD	1.15.1.1	0.38	0.54	0.71	0.76	0.79	0.84	0.88	0.9	0.92																	
GDH	1.4.1.2	0.04	0.09																								
POD (A)	1.11.1.7	0.02	0.03	0.08	0.12	0.21	0.25	0.3	0.4	0.43	0.44	0.48	0.63	0.69	0.73	0.88	0.83	0.92									
POD (C)		0.02	0.07	0.12	0.17	0.25	0.42	0.5	0.7	0.8	0.87																

Table 4.11: Banding pattern of *Pennisetum* species based on Esterase enzyme system.

Sample no.	Band no. →	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
	RM→	0.03	0.08	0.13	0.16	0.18	0.19	0.22	0.24	0.29	0.32	0.35	0.36	0.41	0.42	0.43	0.44	0.46	0.57	0.6	0.61	0.62	0.66	0.7	0.72	0.74	0.76
	Species↓																										
1	*P. squamulatum* IG 2000-36						2+	2+						3+	3+			3+		3+				3+	3+	3+	3+
2	*P. orientale* IP 21951									1+	1+	1+								1+						4+	
3	*P. orientale* IP 22186									1+	1+	1+								1+						3+	
4	*P. orientale* IG 04-165									1+	1+	1+						4+		1+					2+	3+	
5	*P. setaceum* IP 21949			1+	1+	1+					1+	1+		1+	1+			1+		1+				1+	1+	1+	
6	*P. polystachyon* IP 22102											1+												1+	1+	1+	
7	*P. polystachyon* IP 22109																								4+	1+	
8	*P. polystachyon* IP 22121					2+							2+	2+	2+								3+	1+	1+	1+	2+
9	*P. divisum* IP 21957									1+				2+								1+			3+		
10	*P. divisum* IP 21963											1+										1+			3+	3+	3+
11	*P. villosum* IP 21945									1+				3+				2+			2+				4+		
12	*P. squamulatum* IG 98-360						2+	2+						3+	3+					3+				3+	3+	3+	3+
13	*P. flassidum* IP 22195								2+	1+	2+	2+			1+								3+	3+	3+	3+	3+
14	*P. flassidum* IP 22200								2+	1+	2+	2+			1+								3+	3+	3+	3+	3+
15	*P. flassidum* IP 22188								2+	1+	2+	2+			1+								3+	3+	3+	3+	3+
16	*P. hoheneckeri* IP 21954																						2+	1+	1+		
17	*P. hoheneckeri* IP 21953											2+	1+										4+				
18	*P. hoheneckeri* IP 21952											2+	1+										4+				
19	*P .setosum* IP 21942																							3+	1+		
20	*P. clendenstemum* IG 04-166																							2+	3+	2+	
21	*P. pedicellatum* NATP D-1															1+								1+	3+	2+	
22	*P. pedicellatum* IP 21971														3+										3+	2+	
23	*P. pedicellatum* IP 22095															1+	1+						1+	1+	1+	1+	
24	*P. ramosum* IP 22137	2+	4+	2+							1+				1+												
25	*P. ramosum* IP 21935	2+	4+	2+							1+														1+		
26	*P. violaceum* IP 21634				1+			1+			1+								3+				1+		1+	1+	
27	*P. violaceum* IP 21532							1+											3+				1+				
28	*P. violaceum* IP 21534							1+			1+								1+	1+	1+		1+				
29	*P. mollisimum* IP 21782							1+			1+											2+	2+				
30	*P. schweinfurthii* IP 21929			1+								1+		2+	3+				1+			1+			3+	3+	
31	*P. schweinfurthii* IP 21214														3+										3+	3+	
32	*P. schweinfurthii* PS 233 (X)																								3+	3+	
33	*P. schweinfurthii* PS 233 4x(X)																										
34	*P. schweinfurthii* PS 233 4x(OP) a																							1+			
35	*P. schweinfurthii* PS 233 4x(OP) b																							3+			
36	*P. glaucum* IG 99-748 (or T1)									1+	1+									3+	1+				2+		
37	*P. glaucum* IG 2000-01 (or T2)									1+	1+									3+			1+	3+	3+		
38	*P. squamulatum* IG 98-361						2+	2+						3+	3+			3+		3+				3+	3+	3+	3+

1+, 2+, 3+, etc. represents the intensity of the bands.

Table 4.12: Banding pattern of *Pennisetum* species based on Superoxidedesmutase enzyme system.

Sample no.	Band no. →	1	2	3	4	5	6	7	8	9
	RM→	0.38	0.54	0.71	0.76	0.79	0.84	0.88	0.83	0.92
	Species↓									
1	*P. squamulatum* IG 2000-36	1+						3+	3+	3+
2	*P. orientale* IP 21951	3+						3+		
3	*P. orientale* IP 22186	3+						3+		
4	*P. orientale* IG 04-165	3+				3+	3+	3+		
5	*P. setaceum* IP 21949	3+	3+					3+		
6	*P. polystachyon* IP 22102	3+				3+				
7	*P. polystachyon* IP 22109	3+		1+	1+	3+				
8	*P. polystachyon* IP 22121	3+		1+	1+	3+				
9	*P. divisum* IP 21957	3+						3+		
10	*P. divisum* IP 21963	3+						3+		
11	*P. villosum* IP 21945	3+				3+	3+	3+		
12	*P. squamulatum* IG 98-360	1+						3+	3+	3+
13	*P. flassidum* IP 22195	3+				3+	3+	3+		
14	*P. flassidum* IP 22200	3+				3+	3+	3+		
15	*P. flassidum* IP 22188	3+				3+	3+	3+		
16	*P. hoheneckeri* IP 21954		3+					3+		
17	*P. hoheneckeri* IP 21953	3+						3+		
18	*P. hoheneckeri* IP 21952	3+						3+		
19	*P. setosum* IP 21942	1+				3+				
20	*P. clendenstenum* IG 04-166	1+		3+	3+	3+				
21	*P. pedicellatum* (NATP D-1)	1+		3+	3+	3+				
22	*P. pedicellatum* IP 21971	1+		3+	3+	3+				
23	*P. pedicellatum* IP 22095	1+				3+				
24	*P. ramosum* IP 22137	2+						3+		
25	*P. ramosum* IP 21935							3+		
26	*P. violaceum* IP 21634	2+						3+		
27	*P. violaceum* IP 21532	2+						3+		
28	*P. violaceum* IP 21534	2+						3+		
29	*P. mollisimum* IP 21782	2+						3+		
30	*P. schweinfurthii* IP 21929							3+		
31	*P. schweinfurthii* IP 21214							3+		
32	*P. schweinfurthii* PS 233 2x(X)							3+		
33	*P. schweinfurthii* PS 233 4x(X)							3+		
34	*P. schweinfurthii* PS 233 4x(OP) a							3+		
35	*P. schweinfurthii* PS 233 4x(OP) b							3+		
36	*P. glaucum* IG 99-748 (or T1)	3+						3+		
37	*P. glaucum* IG 2000-01 (or T2)	3+						3+		
38	*P. squamulatum* IG 98-361	1+						3+	3+	3+

1+, 2+, 3+, etc. represents the intensity of the bands, X= self pollinated, OP= open pollinated.

Table 4.13a: Banding pattern of *Pennisetum* species based on Peroxidase enzyme system (Anodal bands).

Sample no.	Band no. →	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
	RM→	0.02	0.03	0.08	0.12	0.21	0.25	0.3	0.4	0.43	0.44	0.48	0.63	0.69	0.73	0.8	0.83	0.92
	Species↓																	
1	*P. squamulatum* IG 98-361		1+		3+						3+				3+	1+		
2	*P. violaceum* IP 21579		2+	3+								3+			1+	1+		
3	*P. violaceum* IP 21634			2+								1+				1+		
4	*P. violaceum* IP 21524		2+	2+								1+				3+		
5	*P. violaceum* IP 21532		3+	2+								2+			1+	1+		
6	*P. violaceum* IP 21634		1+									3+				3+		
7	*P. violaceum* PV 2433			3+								1+				1+		
8	*P. glaucum* 81A				4+							1+				1+		
9	*P. glaucum* 81B		3+									1+				1+		
10	*P. glaucum* IG 2000-01 (= T2)		2+	3+								1+				1+		
11	*P. glaucum* IG 99-748 (= T1)			3+								1+			1+	1+		
12	Unidentified species					1+	1+	1+	2+			1+					3+	
13	*P. schweinfurthii* PS 2116			4+	4+	1+												
14	*P. schweinfurthii* IP 21931			4+	4+													
15	*P. schweinfurthii* IP 21929			4+	4+	1+	1+											
16	*P. schweinfurthii* PS 237	1+		4+	4+													
17	*P. mollisimum* IP 21782		2+	3+	1+						3+						2+	
18	*P. ramosum* IP 22180		3+		1+		2+		2+	1+			3+	2+			1+	
19	*P. ramosum* IP 21935		3+		1+		2+			1+			3+	2+			1+	
20	*P. ramosum* IP 22137		3+		1+		2+			1+			3+	2+			1+	
21	*P. divisum* IP 21962				2+	2+			3+				2+				1+	
22	*P. flassidum* IP 22195		2+	4+	1+						2+	2+		2+		2+		
23	*P. squamulatum* IG 98-360			3+	3+	3+					3+				1+	1+		
24	*P. squamulatum* IG 2000-36				1+	1+	3+		3+						1+			1+
25	*P. setaceum* IP 21949		4+	4+				1+				3+			3+		1+	
26	*P. setaceum* IP 21949 IPK				1+			1+		1+					3+		2+	
27	*P. orientale* IG 04-165				1+			1+		1+			2+	2+				
28	*P. orientale* IP 21951				1+	1+	1+	1+		1+			2+	2+			3+	
29	*P. pedicellatum* Agros 4				1+	1+	1+		1+		1+						1+	
30	*P. pedicellatum* IP 21883				1+	1+	1+		1+			1+					1+	
31	*P. pedicellatum* IP 21890				1+	1+	1+		1+								1+	
32	*P. pedicellatum* IP 21879						1+	2+									1+	
33	*P. pedicellatum* IP 21790						2+	2+									1+	
34	*P. pedicellatum* IP 22095					2+			2+				2+				1+	
35	*P. pedicellatum* IP 21971						3+	3+									3+	
36	*P. polystachyon* IP 21900					1+		1+	1+			1+			1+		3+	
37	*P. polystachyon* IP 21904					1+		1+	1+			2+			1+		3+	
38	*P. polystachyon* IP 22121					1+		1+	1+			1+			1+		3+	
39	*P. polystachyon* IP 22102				3+	3+		1+	1+			1+					3+	
40	*P. pedicellatum* NATP D-1					3+		1+	3+	1+		2+	2+		1+		3+	
41	*P. polystachyon* IP 21902							3+	3+						1+		3+	1+

1+, 2+, 3+, etc. represents the intensity of the bands.

Table 4.13b: Banding pattern of *Pennisetum* species based on Peroxidase enzyme system (Cathodal bands).

Sample no.	Band no. →	1	2	3	4	5	6	7	8	9	10
	RM→	0.02	0.07	0.12	0.17	0.25	0.42	0.5	0.7	0.8	0.87
	Species↓										
1	*P. squamulatum* IG 98-361	2+				3+					1+
2	*P. violaceum* IP 21579	2+				1+		2+			1+
3	*P. violaceum* IP 21634	2+				1+					1+
4	*P. violaceum* IP 21524	2+				1+		2+			1+
5	*P. violaceum* IP 21532	1+				1+		2+			1+
6	*P. violaceum* IP 21634	1+				1+					
7	*P. violaceum* PV 2433					1+					1+
8	*P. glaucum* 81A	1+				1+		2+			1+
9	*P. glaucum* 81B	1+									1+
10	*P. glaucum* IG 2000-01 (or T2)					1+					1+
11	*P. glaucum* IG 99-748 (or T1)					1+					1+
12	Unidentified species	1+						1+	1+		
13	*P. schweinfurthii* PS 2116										1+
14	*P. schweinfurthii* IP 21931										2+
15	*P. schweinfurthii* IP 21929										2+
16	*P. schweinfurthii* PS 237										2+
17	*P. mollisimum* IP 21782	1+					3+		1+		2+
18	*P. ramosum* IP 22180						1+				2+
19	*P. ramosum* IP 21935						1+				2+
20	*P. ramosum* IP 22137						1+			2+	2+
21	*P. divisum* IP 21962										
22	*P. flassidum* IP 22195	1+				1+		3+	3+		3+
23	*P. squamulatum* IG 98-360					2+					1+
24	*P. squamulatum* IG 2000-36		2+			3+					2+
25	*P. setaceum* IP 21949	2+		2+							2+
26	*P. setaceum* IP 21949 IPK	2+		2+		3+					2+
27	*P. orientale* IG 04-165	2+		2+		3+		3+		1+	2+
28	*P. orientale* IP 21951	2+		2+		3+	2+			1+	2+
29	*P. pedicellatum* Agros 4		2+		2+						
30	*P. pedicellatum* IP 21883		2+		2+						
31	*P. pedicellatum* IP 21890		2+		2+						
32	*P. pedicellatum* IP 21879										
33	*P. pedicellatum* IP 21790										
34	*P. pedicellatum* IP 22095					3+				1+	
35	*P. pedicellatum* IP 21971				2+	2+					
36	*P. polystachyon* IP 21900	1+									
37	*P. polystachyon* IP 21904		2+								
38	*P. polystachyon* IP 22121		2+								
39	*P. polystachyon* IP 22102		2+								1+
40	*P. pedicellatum* NATP D-1		2+						1+	1+	
41	*P. polystachyon* IP 21902		2+						1+	1+	

1+, 2+, 3+, etc. represents the intensity of the bands.

Table 4.14: Banding pattern of *Pennisetum* species based on Glutamate dehydrogenase enzyme system.

Sample no.	Band no. →	1	2
	RM→	0.04	0.09
	Species↓		
1	*P. squamulatum* IG 98-360	2+	
2	*P. violaceum* IP 21634	2+	
3	*P. violaceum* IP 21532	2+	
4	*P. mollisimum* IP 21782	2+	
5	*P. glaucum* IG 99-748 (= T1)	2+	
6	*P. glaucum* IG 2000-01 (= T2)	2+	
7	*P. schweinfurthii* PS 233 4x (X)	1+	
8	*P. schweinfurthii* PS 233 4x(OP)a	1+	
9	*P. schweinfurthii* PS 233 4x(OP)b	1+	
10	*P. squamulatum* IG 98-361	2+	
11	*P. flassidum* IP 22195	2+	
12	*P. flassidum* IP 22188	2+	
13	*P. hoheneckeri* 21953	2+	
14	*P. hoheneckeri* IP 21952	2+	
15	*P. clendenstenum* IG 04-166		2+
16	*P. pedicellatum* NATP D-1	2+	
17	*P. polystachyon* IP 22102	2+	
18	*P. pedicellatum* IP 21971	2+	
19	*P. pedicellatum* IP 22095	2+	
20	*P. squamulatum* IG 2000-36	2+	
21	*P. setaceum* IP 21949	2+	
22	*P. orientale* IP 21951	1+	
23	*P. orientale* IP 22186	1+	
24	*P. setosum* IP 21942		2+

1+, 2+, 3+, etc. represents the intensity of the bands.
X= self pollinated, OP= open pollinated.

Table 4.15: Details of application of colchicine treatments.

Accession no.	Treatments	No. of seeds/plantlets treated per accession (approx. values)	Success (%)
P. glaucum (81A1, 81A4, 81A5, IG 99-748 or T1)	1. Seed treatment 2. Shoot treatment 3. Seedling capillary treatment 4. Colchicine injection	50 50 30 10	- 4% (2 induced tetraploids (81A1 and 81A4)) - -
P. violaceum (IP 21586, IP 21579, IP 21532, IP 21641, IP 21634, IP 21632, IP 21634)	1. Seed treatment 2. Shoot treatment 3. Seedling capillary treatment	100 50 50	- - -
P. schweinfurthii (PS 233)	1. Seed treatment 2. Shoot treatment 3. Seedling capillary treatment	100 50 20	- - 5% (1 induced tetraploid)
P. ramosum (IP 22137, IP 21935, IP 22180)	1. Seed treatment 2. Seedling capillary treatment	100 50	- -

Table 4.16: Cytological observations on diploid and induced tetraploid *P. schweinfurthii.*

S.no	Species	Accession no.	Chromosome no. (2n)	Ploidy	PMC studied	Chromosomal association and range* at diakinesis				Chiasmata frequency (%) & Range*	Pollen stainability (%)
						I	II	III	IV		
1.	***P. schweinfurthii***	PS 233	14	2x	15	0.26	6.86	-	-	9.8	96
						(0-2)	(6-7)			(9-12)	
2.	***P. schweinfurthii***	PS 233 (4x)	28	4x	20	0.95	10.3	0.35	1.35	17.55	80.1
						(0-3)	(7-14)	(0-2)	(0-2)	(13-25)	

*Figure in parenthesis denotes range.

Table 4.17: Morphological data (metric traits) of diploid and induced tetraploid *P. glaucum.*

S.no.	Species	Accession no.	No. of Tillers	Height of main tiller (cm)	No. of leaves/ tiller	Flag leaf		3rd leaf		Stem Girth (cm)	No. of nodes/ tiller	Internode length (cm)	Peduncle length (cm)	Spike (cm)		No. of Florets/ spikelet
						Length (cm)	Width (cm)	Length (cm)	Width (cm)					Length (cm)	Width (cm)	
1	*P. glaucum*	81A1 (2x)	3	115	12	26	2	38	3	1	5	12	30	22	3	2
2	*P. glaucum*	81A4 (2x)	2.67	119.67	13.67	33.67	3.17	36.67	3.47	0.97	8.33	11.33	27	19.33	1.63	2
3	*P. glaucum*	IG 99-748 (= T1)	1.33	84.67	10	35	3.5	66.67	4.37	1.43	5.33	11	18.33	20	2.8	2
4	*P. glaucum* (C_0)	81A1 (4x)	7	52	7	16	1	30	1	0.5	4	9	11	12	1.5	2
5	*P. glaucum* (C_0)	81A4 (4x)	7	60	6	30	2	40	2	0.4	4	9	21	11.5	1.5	2
6	*P. glaucum* (C_1)	81A1 (4x)	7	52	7	16	1	30	1	0.5	4	9	11	12	1.5	2
7	*P. glaucum* (C_1)	81A4 (4x)	1	104	14.67	18.67	3.33	46	4.17	1.3	5	13.67	36.67	24.67	2.5	2

Table 4.18: Morphological data (qualitative characters) of diploid and induced tetraploid *P. glaucum*

S.no.	Species	Accession no.	Node hairiness	Coloured ring at node	Leaf base		Stigma colour
					colour	hairiness	
1	*P. glaucum*	81A1 (2x)	NH	Y	Y	H	W
2	*P. glaucum*	81A4 (2x)	NH	Y	Y	H	W
3	*P. glaucum*	IG 99-748 (= T1)	H	Y	Y	H	W
4	*P. glaucum* (C_0)	81A1 (4x)	NH	Y	Y	H	W
5	*P. glaucum* (C_0)	81A4 (4x)	NH	Y	Y	H	W
6	*P. glaucum* (C_1)	81A1 (4x)	NH	Y	Y	H	W
7	*P. glaucum* (C_1)	81A4 (4x)	NH	Y	Y	H	W

NH=Non hairy, H= Hairy, Y= Yellow, W= white.

Table 4.19: Cytological observations on diploid and induced tetraploid *P. glaucum.*

S.no	Species	Accession no.	Chromosome no. (2n)	Ploidy	PMC studied	Chromosomal association and range* at diakinesis				Chiasmata frequency (%) & Range*	Pollen Stainability (%)
						I	II	III	IV		
1.	*P. glaucum*	81A1	14	2x	30	-	7	-	-	13.9	sterile
										(11-16)	
2.	*P.glaucum*	81A4	14	2x	30	-	7	-	-	13.6	sterile
										(12-14)	
3	*P.glaucum*	81A1	28	4x	20	0.8	11.05	0.7	0.75	20.35	sterile
						(0-5)	(6-14)	(0-2)	(0-3)	(16-24)	
4	*P.glaucum*	81A4	28	4x	25	0.04	11.16	0.12	1.32	25.04	sterile
						(0-1)	(8-14)	(0-2)	(0-3)	(18-32)	

*Figures in parenthesis denote range.

Table 4.20: Crosses attempted in the study.

Year	Cross	No. of spikes pollinated	No. of seeds obtained/spike	No. of hybrids obtained
2003-04	*P. glaucum* (MS) x *P. violaceum* (PV 2433)	10	60	20
	P. glaucum (MS) x *P. mollisimum* (IP 21782)	12	60	20
	P. glaucum (MS) x *P. schweinfurthii* (IP 24214)	6	-	-
	P. glaucum (MS) x *P. ramosum* (IP 22180)	8	1	-
	x *P. ramosum* (IP 22137)	4	-	-
	x *P. ramosum* (IP 21935)	30	11	-
	P. glaucum (MF) x *P. ramosum* (IP 21935)	2	-	-
	P. glaucum (T1) x *P. schweinfurthii* (IP 24214)	8	-	-
	x *P. schweinfurthii* (IP 21929)	4		
	P. glaucum (T1) x *P. ramosum* (IP 21935)	6	-	-
	P. glaucum (T1) x *P. orientale* (IG 04-165)	16	-	-
	P. glaucum (T1) x *P. setaceum* (IP 21949)	2	-	-
	P. glaucum (T1) x *P. flassidum* (IP 22195)	6	-	-
	P. glaucum (T1) x *P. polystachyon* (IP 22102)	8	-	-
	x *P. polystachyon* (IP 22109)	4	20	-
	P. glaucum (T1) x *P. pedicellatum* (Agros 4)	20	10	-
	x *P. pedicellatum* (IP 21890)	4	-	
	x *P. pedicellatum* (IP 21790)	2		
	x *P. pedicellatum* (IP 21971)	6		
	x *P. pedicellatum* (IP 22095)	8		
	x *P. pedicellatum* (NATP D-1)	16	10 (shrivelled)	
	P. glaucum (T1) x *P. squamulatum* (IG 98-360)	6	20	
	x *P. squamulatum* (IG 2000-36)	26	20	3
	P. schweinfurthii (IP 21929) x *P. ramosum* (IP 22180)	2	-	-
	P. schweinfurthii (IP 24214) x *P. violaceum* (PV 2433)	4	-	-
	P. schweinfurthii (IP 21929) x *P. glaucum* (MS)	2	-	-
	x *P. glaucum* (T1)	2	-	-
	P. ramosum (IP 22180) x *P. glaucum* (T1)	6	-	-
	P. glaucum (T1) x H1	4	10	27
	x H2	10	10	15
	BC_1 (GO) x *P. glaucum* (T1)	22	Fluff	3
	x *P. orientale* (IG 04-165)	4	-	-
	x *P. schweinfurthii* (IP 24214)	6	-	-
	x *P. pedicellatum* (Agros 4)	6	-	
	x H1	4	Fluff	1
	x H2	12	Fluff	4
	P. glaucum (MS) x H1	24	1	-
	x H2	56	1	-
	BC_1 (H1T2) x *P. polystachyon* (IP 22121)	2	-	-
	x *P. schweinfurthii* (IP 21929)	4	-	-
	BC_1 T2H1) x *P. violaceum* (PV 2433)	2	Fluff	-
	BC_1 (T1H1) x *P. glaucum* (T1 and T2)	40	40	-
2004-05	*P. glaucum* (T1) x *P. orientale* (IG 04-165)	4	20	-
	P. glaucum (MS) x *P. orientale* (IP 21951)	2	20 (shrivelled)	-
	P. glaucum (T1) x *P. polystachyon* (IP 22102)	10	10	-
	x *P. polystachyon* (IP 22109)	2	10	-
	x *P. polystachyon* (IP 22121)	2	10	-
	P. glaucum (T1) x *P. pedicellatum* (Agros 4)	24	25	-
	x *P. pedicellatum* (IP 22095)	6	20	-
	P. glaucum (T1 or T2) x BC_1 T2H2	5	30	
2005-06	*P. glaucum* (MS) x *P. orientale* (IP 21951)	1	-	-
	P. glaucum (MF) x *P. pedicellatum* (Agros 4)	1	-	-
	P. glaucum (T1) x BC_1 H1T1	1	50	
	P. glaucum (T1) x BC_1 T2H1	1	50	

MS= male sterile lines, MF= male fertile lines, T1= IG 99-748, T2= IG 2000-01, H1= sexual F_1 hybrid (T1x *P. squamulatum* IG 98-360), H2= apomictic F_1 hybrid (T1x *P. squamulatum* IG 98-360), H1T2= H1xT2, T2H1= T2xH1, T1H1= T1xH1, T2H2=T2xH2, H1T1= H1xT1, BC_1 (GO)= hybrid between F_1 (*P. glaucum* x *P. orientale*) and *P. glaucum* (2x)

Table 4.21: Results from interspecific crosses of *P. glaucum* and other wild species following embryo rescue.

	Control *P. glaucum* T1* and T2*	*P. polystachyon* IP 22109	*P. polystachyon* IP 22102	*P. pedicellatum* IP 22095 (4x)	*P. pedicellatum* Agros 4 (8x)
Spikes pollinated	10	20	20	30	30
Embryos cultured	30	150	50	150	150
Hybrids transferred to the field	-	-	-	-	-

*T1= IG 99-748, T2= IG 2000-01

Table 4.22: Morphological data (metric traits) of pearl millet *x P. violaceum* and pearl millet *x P. mollisimum*.

S.no.	Name	Accession no.	No. of tillers	Height. of main tiller (cm)	No. of leaves/ tiller	Flag leaf		3rd leaf		Stem girth (cm)	No. of nodes/ tiller	Internode length (cm)	Peduncle length (cm)	Spike	
						length (cm)	width (cm)	length (cm)	width (cm)					length (cm)	width (cm)
1	***P. glaucum***	81A1	8	110	12	26	2.5	40	3	1	5	13	30	26	3
2	***P. violaceum***	PV 2433	7.67	68.33	6.67	11.67	0.4	21.67	0.87	0.27	6.67	13.67	11.67	6.67	1.13
3	***P. glaucum* x *P. violaceum***	F_1	7	131.67	7	16.67	0.93	45	1.67	0.5	7	14.33	24.33	13.33	1.43
4	***P. glaucum* x *P. violaceum***	F_2	2	86.67	6.33	15	1	33.33	1.57	0.3	6.33	13	20.67	8	1.67
5	***P. glaucum* x *P. violaceum***	BC_1	4	110	7.33	21	2	50	2.3	0.7	7.33	12.67	26	17.33	1.43
6	***P. glaucum* x *P. mollisimum***	F_1	6	240	10.33	14.33	1.27	26.33	2.06	0.63	10.33	21	26.67	16	1.67

Table 4.23: Morphological characters (range, mean and standard deviation) in F_1, F_2, and BC_1 population.

S.no.	Name	Accession no./generation		No. of tillers	Height of main tiller (cm)	No. of leaves/ tiller	Flag leaf		3rd leaf		Stem girth (cm)	No. of nodes/ tiller	Internode length (cm)	Peduncle length (cm)	Spike	
							length (cm)	width (cm)	length (cm)	width (cm)					length (cm)	width (cm)
1	***P. glaucum***	81A1	Mean	8	110	12	26	2.5	40	3	1	5	13	30	26	3
2	***P. violaceum***	PV 2433	Mean	7.67	68.33	6.67	11.67	0.40	21.67	0.87	0.27	6.67	13.67	11.67	6.67	1.13
3	***P. mollisimum***		Range	3-12	230-310	12-16	17-28	1.0	35-55	1.2-2.4	0.5-1.1	9-15	17.5-27	15-22	13-16	1.5-2.2
			Mean	8.67	280.00	13.33	24.00	1.00	45.00	1.63	0.72	11.67	21.83	18.67	14.67	1.77
			SD	4.93	43.59	2.31	6.08	0.00	10.00	0.67	0.33	3.06	4.80	3.51	1.53	0.38
4	***P. glaucum* x *P. violaceum***	F_1	Range	4-10	110-145	6-8	15-20	0.8-1.0	40-50	1.5-2.0	0.4-0.6	6-8	14-15	23-25	12-14	1.3-1.5
			Mean	7.00	131.67	7.00	16.67	0.93	45.00	1.67	0.50	7.00	14.33	24.33	13.33	1.43
			SD	3.00	18.93	1.00	2.89	0.12	5.00	0.29	0.10	1.00	0.58	1.15	1.15	0.12
5	***P. glaucum* x *P. violaceum***	F_2	Range	1-3	70-100	6-7	10-20	1.0	25-40	1.4-1.8	0.3	6-7	10-15	17-27	7-9	1.5-2.0
			Mean	2.00	86.67	6.33	15.00	1.00	33.33	1.57	0.30	6.33	13.00	20.67	8.00	1.67
			SD	1.00	15.28	0.58	5.00	0.00	7.64	0.21	0.00	0.58	2.65	5.51	1.00	0.29
6	***P. glaucum* x *P. violaceum***	BC_1	Range	1-7	90-140	6-8	18-25	2-2.5	40-55	1.5-2.8	0.6-0.8	6-8	11-14	20-30	11-21	1.3-1.5
			Mean	4.00	110.00	7.33	21.00	2.00	50.00	2.30	0.70	7.33	12.67	26.00	17.33	1.43
			SD	3.00	26.46	1.15	3.61	0.50	8.66	0.70	0.10	1.15	1.53	5.29	5.51	0.12
7	***P. glaucum* x *P. mollisimum***	F_1	Range	4-8	200-310	10-11	12.5-17	1-1.5	23-30	1.7-2.5	0.6-0.69	9-12	15-28	23-32	11-19	1.5-2.0
			Mean	6.00	240.00	10.33	14.33	1.27	26.33	2.07	0.63	10.33	21.00	26.67	16.00	1.67
			SD	2.00	60.83	0.58	2.36	0.25	3.51	0.40	0.05	1.53	6.56	4.73	4.36	0.29

Table 4.24: Morphological data (qualitative traits) of pearl millet *x P. violaceum* and pearl millet *x P. mollisimum*.

S.no.	Name	Accession no./generation	Node hairiness	Coloured ring at node	Leaf base		Stigma colour	Awn colour
					colour	hairiness		
1	***P. glaucum***	81A1	NH	V	LV	H	W	Y
2	***P. violaceum***	PV 2433	H	DV	V	H	W	V
3	***P. glaucum* x *P. violaceum***	F_1	H	V	V	H	W	V
4	***P. glaucum* x *P. violaceum***	F_2	H	V	Y+V	H	W	V
5	***P. glaucum* x *P. violaceum***	BC_1	H+NH	V	Y	H	W	V
6	***P. glaucum* x *P. mollisimum***	F_1	H	DV	DV	H	W	V

NH=non hairy, H= hairy, Y= yellow, V= violet, DV= dark violet, LV= light violet, W= white.

Table 4.25: Pollen of pearl millet x *P. violaceum* F_1 hybrids.

S. no.	Species	Accession no.	Pollen		
			Stainability (%)	Mean (µm)	Size (µm)
1	*P. glaucum*	81A1	sterile	-	-
2	*P. violaceum*	PV 2433	98.3	46.4	30-60
3	F_1 hybrid	20/1	85.0	36.5	24-45
4		20/9	85.6	35.1	21-45
5		20/4	91.7	45.7	30-60
6		20/2	93.2	42.8	30-60
7		20/10	95.0	36.5	21-48

Table 4.26: Nomenclature of hybrids (pearl millet x *P. squamulatum*).

Generation	Cross	Plant no./name
F_1	T1MA	H1
	T1MA	H2
	T1MC	H3
	T2MC	H4
	T2MC	H5
BC_1	H1T1	MP 5/6, MP 5/8, MP 5/10, MP 5/11, MP 5/12, MP 5/13, MP 5/14, MP 5/15, MP 5/16, MP 5/17, MP 5/18, MP 5/19, MP 5/20, MP 5/21, MP 5/23, MP 5/24, MP 2/29
	T1H1	MP 2/11, MP 2/12, MP 2/14, MP 2/15, MP 2/16, MP 2/31, MP 5/29, MP 5/30, MP 5/31, MP 5/32, MP 5/34, MP 5/35, MP 5/36, MP 5/37, MP 5/39, MP 5/40, MP 5/41
	H1T2	MP 5/26, MP 5/27
	T2H1	MP 2/17, MP 2/18, MP 2/32, MP 4/3, MP 4/4, MP 4/6, MP 4/7, MP 4/8, MP 4/30, MP 4/31.
	T2H2	MP 2/23, MP 2/24, MP 2/25, MP 2/26, MP 2/27, MP 4/12, MP 4/14, MP 4/15, MP 4/17, MP 4/33, MP 4/34.
	T1H2	MP 2/20, MP 2/21, MP 4/10, MP 4/32.

T1= *P. glaucum* IG 99-748, T2= *P. glaucum* IG 2000-01, MA= *P.squamulatum* IG 98-360, MC= *P. squamulatum* IG 2000-36, H1T1, T1H1,T1H2 etc. denotes crosses between H1and T1, T1 and H1, T1and H2 respectively and so on.

Table 4.27: Grouping of plants (pearl millet x *P. squamulatum* F_1 hybrids and BC_1 plants) in different clusters based on morphological characters.

Cluster no.	Total no. of plants	
1	10	H2, H1T1 (MP 5/11, MP 5/24, MP 2/29), H1T2 (MP 5/27), T1H1 (MP 5/29, MP 5/31, MP 2/12, MP 2/16,) T2H1 (MP 2/18)
2	7	T2H1 (MP 4/3), T2H2 (MP 4/17, MP 2/24, MP 2/25, MP 2/26, MP 2/27), T1H2 (MP 2/21)
3	6	H1, T1H1 (MP 5/39, MP 5/40, MP 5/41, MP 2/31), T2H1 (MP 2/32)
4	2	*P. glaucum* (T1, T2)
5	11	T1H1 (MP 5/32, MP 5/34, MP 2/11, MP 2/14, MP 2/15), T2H1 (MP 4/31, MP 4/8, MP 2/17), T2H2 (MP 4/12), T1H2 (MP 4/32, MP 2/20)
6	1	*P .squamulatum* IG 98-360 (or MA)
7	19	H1T1 (MP 5/6, MP 5/8, MP 5/10, MP 5/12, MP 5/13, MP 5/14, MP 5/16, MP 5/17, MP 5/18, MP 5/19, MP 5/20, MP 5/21), T1H1 (MP 5/30, MP 5/36, MP 5/37), T2H1 (MP 4/4, MP 4/6), T2H2 (MP 4/14), T1H2 (MP 4/10)
8	10	H1T1 (MP 5/15, MP 5/23), H1T2 (MP 5/26), T1H1 (MP 5/35), T2H1 (MP 4/30, MP 4/7), T2H2 (MP 4/15, MP 4/33, MP 4/34, MP 2/23)

T1= IG 99-748, T2= IG 2000-01, H1 and H2= F_1 hybrids between pearl millet and *P. squamulatum* (IG 98-360)
H1T1, T1H1, T1H2 etc. denotes the BC_1 crosses of H1and T1, T1 and H1, T1and H2 respectively and so on.

Table 4.28: Distance between cluster centroids (pearl millet x *P. squamulatum* F_1 hybrids and BC_1 plants).

Clusters	1	2	3	4	5	6	7	8
1	0.000							
2	4.310	0.000						
3	2.642	6.092	0.000					
4	8.603	8.186	10.325	0.00				
5	2.408	3.259	4.018	8.456	0.00			
6	9.611	8.981	11.672	13.081	9.309	0.00		
7	2.608	3.973	4.118	7.585	1.898	9.929	0.00	
8	5.410	3.649	7.305	6.874	3.754	9.131	3.471	0.00

Table 4.29: Morphological characters (range, mean and standard deviation) in pearl millet x *P. squamulatum* derived F_1 hybrids and BC_1 plants.

S. no.	Accession/cross		No. of Tillers	Height of main tiller(cm)	Peduncle length (cm)	Spike		No. of leaves	Flag Leaf		3rd Leaf		Stem girth (cm)	No. of nodes	Internode length (cm)
						length (cm)	width (cm)		length (cm)	width (cm)	length (cm)	width (cm)			
1	*P. squamulatum* IG 98-360	Mean	106.67	186.33	57.00	31.67	2.00	10.67	31.33	1.40	68.67	1.60	0.57	10.67	16.00
		SD	3.61	18.72	3.46	0.58	0.00	0.58	2.52	0.20	2.52	0.26	0.06	0.58	5.29
2	*P. glaucum* IG 99-748 (= T1)	Mean	4.46	136.67	28.00	22.33	1.13	6.33	42.70	3.5	71.7	3.67	1.67	6	15.33
		SD	2.52	10.41	7.64	4.04	0.12	0.58	0.58	0.58	0.58	0.58	0.58	0.58	0.58
3	*P. glaucum* IG 2000-01 (= T2)	Mean	1.33	140.00	25.00	23.33	3.00	8.33	26.33	2.93	48.33	4.00	1.33	5.33	16.67
		SD	0.58	36.06	3.00	4.16	0.00	0.58	1.15	0.12	0.58	0.00	0.15	2.08	0.58
4	H1	Mean	3.00	80.00	30.67	19.00	1.60	6.00	16.67	0.90	43.17	1.03	0.50	5.33	11.67
		SD	2.65	25.51	2.52	1.73	0.17	1.00	3.06	0.10	3.88	0.15	0.10	1.15	2.02
5	H2	Mean	15.33	104.00	30.33	26.33	1.93	8.67	22.83	1.07	44.33	1.23	0.63	7.00	7.83
		SD	2.52	14.93	7.77	3.06	0.60	1.15	3.25	0.15	3.21	0.25	0.12	1.00	0.29
6	H1T1	Range	4.33-28.33	44.62-171.00	15.29-42.67	10.81-27.00	1.47-2.45	3.66-8.00	9.78-33.33	0.46-2.30	16.92-56.33	0.58-2.37	0.29-0.83	3.00-7.67	2.80-18.33
		Mean	10.12	133.54	35.83	22.90	1.92	6.61	23.93	1.54	40.79	1.68	0.62	5.52	14.87
		SD	5.64	28.99	6.49	4.03	0.32	1.08	6.17	0.45	9.44	0.43	0.12	1.07	3.62
7	H1T2	Range	9.50-18.33	119.00-188.00	31.00-54.33	18.50-28.83	1.55-1.87	6.67-8.0	20.75-27.67	1.33-1.40	41.25-57.50	1.50-1.93	0.60-0.70	6.60-7.0	17.75-22.33
		Mean	13.92	153.50	42.67	23.67	1.71	7.33	24.21	1.37	49.38	1.72	0.65	6.75	20.04
		SD	6.25	48.79	16.50	7.31	0.22	0.94	4.89	0.05	11.49	0.31	0.07	0.35	3.24
8	T1H1	Range	1.50-28.0	73.50-174.67	24.0-56.33	14.33-27.0	0.18-1.97	4.0-8.0	7.0-31.50	0.70-1.90	19.25-60.17	0.90-2.03	0.36-0.80	4.67-10.0	4.0-23.67
		Mean	12.38	129.85	35.13	20.50	1.55	6.25	20.35	1.39	39.64	1.66	0.59	6.42	14.82
		SD	8.32	27.12	8.52	3.88	0.18	1.10	5.94	0.35	10.17	0.35	0.11	1.22	5.06
9	T2H1	Range	9.50-22.67	124.67-199.0	29.0-45.83	18.50-33.0	1.3-2.5	6.33-9.33	13.50-30.83	1.25-2.30	34.50-65.0	0.75-3.0	0.55-0.83	6.0-16.83	8.67-19.33
		Mean	15.87	168.80	39.20	25.29	1.85	7.88	23.44	1.72	47.59	1.95	0.67	8.13	14.96
		SD	4.47	27.67	5.96	4.92	0.33	1.02	5.36	0.35	9.70	0.62	0.09	3.14	3.69
10	T2H2	Range	7.67-25.0	146.0-258.67	31.67-61.33	24.50-34.0	1.77-2.8	6.0-11.33	16.27-45.33	1.0-2.27	17.83-39.03	1.27-2.57	0.57-0.97	5.67-10.0	12.83-21.0
		Mean	15.09	211.91	41.91	29.30	2.19	8.58	27.60	1.67	52.15	1.97	0.78	7.73	16.80
		SD	4.84	39.05	5.38	2.77	0.30	1.75	7.42	0.36	10.94	0.42	0.12	1.43	2.48
11	T1H2	Range	12.50-24.67	122.0-202.0	37.61-42.17	24.67-33.33	1.70-2.23	6.0-9.0	16.17-32.33	0.80-1.47	29.33-47.67	1.03-1.53	0.53-0.75	5.0-9.0	14.33-16.33
		Mean	16.38	171.25	39.21	26.96	1.86	7.75	22.21	1.11	39.58	1.24	0.65	7.08	15.70
		SD	5.72	36.09	2.05	4.25	0.25	1.50	7.57	0.30	7.89	0.24	0.09	1.64	0.92

H1 and H2= F_1 hybrids (*P. glaucum* IG 99-748 x *P. squamulatum* IG 98-360)

Table 4.30: Morphological characters (mean±standard deviation) in different clusters of pearl millet x *P. squamulatum* F_1 hybrids and BC_1 plants.

Cluster	1	2	3	4	5	6	7	8	9	10	11	12	13
1	19.58± 7.00	126.90± 16.00	2940± 3.12	19.35± 3.43	1.57± 0.25	7.35± 0.96	20.42± 3.14	1.33± 0.24	39.34± 5.66	1.52± 0.26	0.62± 0.09	6.42± 0.83	14.45± 3.23
2	18.95± 4.20	218.90± 31.27	38.12± 5.32	28.00± 2.03	2.00± 0.20	9.62± 1.15	23.12± 3.69	1.51± 0.27	45.10± 4.88	1.76± 0.34	0.78± 0.09	9.93± 3.16	14.57± 3.55
3	8.94± 7.99	98.53± 25.48	30.28± 3.61	17.88± 1.36	1.46± 0.10	5.58± 1.13	14.53± 4.05	1.04± 0.24	31.38± 7.81	1.15± 0.35	0.49± 0.08	6.64± 1.90	9.19± 3.69
4	3.00± 2.36	138.33± 2.36	26.00± 2.83	21.50± 1.18	2.72± 0.07	7.33± 1.41	34.50± 11.55	3.22± 0.40	60.00± 16.50	3.83± 0.24	1.50± 0.24	5.67± 0.47	16.00± 0.94
5	12.83± 4.68	165.94± 23.07	41.76± 2.63	23.31± 2.21	1.74± 0.27	7.30± 1.11	19.77± 3.04	1.38± 0.35	40.18± 7.15	1.75± 0.40	0.63± 0.07	6.77± 0.76	17.08± 1.88
6	106.67	270.00	45.67	21.0	1.00	10.33	41.67	1.53	56.33	1.57	0.56	7.67	0.26
7	9.33± 4.10	136.75± 14.71	37.81± 2.53	24.90± 3.15	1.97± 0.21	6.44± 0.76	26.37± 4.01	1.60± 0.25	44.85± 5.87	1.71± 0.23	0.64± 0.08	5.61± 0.74	15.38± 1.95
8	12.07± 3.94	194.40± 29.75	46.87± 5.21	29.82± 2.88	2.17± 0.34	7.67± 1.20	31.15± 5.72	1.98± 0.35	59.86± 7.21	2.34± 0.37	0.75± 0.12	7.13± 1.00	19.12± 2.41

1=number of tillers, 2= height of main tiller, 3= peduncle length, 4=spike length, 5= spike width, 6=number of leaves, 7=flag leaf length, 8= flag leaf width, 9= 3rd leaf length, 10= 3rd leaf width, 11= stem girth, 12= number of nodes, 13= internode length.

Table 4.31: Variability estimates for various metric traits in pearl millet x *P. squamulatum* F_1 hybrids and BC_1 plants.

Characters→	1	2	3	4	5	6	7	8	9	10	11	12	13
Range	1.33-106.67	73.50-270.0	24.00-56.33	14.33-34.00	1.00-2.80	4.00-11.33	7.00-45.33	0.70-3.50	19.25-71.67	0.90-4.00	0.47-1.67	4.33-16.83	4.00-29.00
Mean	14.15	156.16	37.67	24.07	1.87	7.25	24.15	1.57	44.95	1.80	0.68	6.74	15.67
CV	37.48	15.97	11.65	13.74	14.77	12.69	21.81	20.99	19.26	18.04	17.10	15.49	15.17
GCV	91.31	26.05	17.18	17.40	17.83	18.87	25.58	27.85	20.40	29.40	25.70	25.17	23.32
PCV	98.70	30.56	20.75	22.17	23.16	22.75	33.62	34.88	28.05	34.49	30.87	29.56	27.82
GA	24.63	71.45	11.03	6.77	0.53	2.34	9.68	0.72	13.73	0.93	0.30	2.98	6.31
Heritability	0.86	0.73	0.69	0.62	0.59	0.69	0.58	0.64	0.53	0.73	0.69	0.73	0.70

1=number of tillers, 2= height of main tiller, 3= peduncle length, 4=spike length, 5= spike width, 6=number of leaves, 7=flag leaf length, 8= flag leaf width, 9= 3rd leaf length, 10= 3rd leaf width, 11= stem girth, 12= number of nodes, 13= internode length.
CV=coefficient of variation, GCV= genotypic coefficient of variation, PCV= phenotypic coefficient of variation, GA= genetic advance values.

Table 4.32: Genotypic correlation coefficient based on morphological observations in the pearl millet x *P. squamulatum* F_1 hybrids and BC_1 plants.

Characters	1	2	3	4	5	6	7	8	9	10	11	12	13
1	1.000												
2	0.460	1.000											
3	0.121	0.615	1.000										
4	-0.101	0.594	0.649	1.000									
5	-0.464	0.297	0.232	0.648	1.000								
6	0.427	0.818	0.201	0.398	0.251	1.000							
7	0.214	0.475	0.369	0.567	0.483	0.240	1.000						
8	-0.130	0.311	0.165	0.363	0.625	0.233	0.689	1.000					
9	0.092	0.505	0.492	0.637	0.494	0.264	0.848	0.647	1.000				
10	-0.182	0.337	0.169	0.339	0.622	0.241	0.617	0.964	0.650	1.000			
11	-0.132	0.361	-0.049	0.284	0.640	0.381	0.631	0.921	0.603	0.867	1.000		
12	0.192	0.445	0.023	0.258	0.025	0.617	-0.089	-0.026	0.025	0.023	0.099	1.000	
13	0.405	0.554	0.654	0.275	0.077	0.200	0.586	0.360	0.549	0.416	0.185	-0.276	1.000

1=Number of tillers, 2= height of main tiller, 3= Peduncle length, 4=spike length, 5= Spike width, 6=number of leaves, 7=Flag leaf length, 8= Flag leaf width, 9= 3rd leaf length, 10= 3rd leaf width, 11= Stem girth, 12= Number of nodes, 13= Internode length.

Table 4.33: Phenotypic correlation coefficient based on morphological observations in the pearl millet x *P. squamulatum* F_1 hybrids and BC_1 plants.

Characters	1	2	3	4	5	6	7	8	9	10	11	12	13
1	1.000												
2	0.391	1.000											
3	0.077	0.580	1.000										
4	-0.082	0.547	0.588	1.000									
5	-0.344	0.274	0.223	0.589	1.000								
6	0.376	0.713	0.229	0.348	0.203	1.000							
7	0.171	0.401	0.314	0.472	0.426	0.199	1.000						
8	-0.111	0.311	0.177	0.356	0.523	0.222	0.715	1.000					
9	0.064	0.434	0.401	0.480	0.394	0.207	0.763	0.597	1.000				
10	-0.126	0.377	0.181	0.353	0.485	0.254	0.550	0.815	0.602	1.000			
11	-0.096	0.349	0.024	0.315	0.536	0.319	0.449	0.657	0.409	0.675	1.000		
12	0.185	0.463	0.111	0.262	0.036	0.593	-0.004	0.066	0.073	0.061	0.119	1.000	
13	0.302	0.480	0.548	0.287	0.125	0.204	0.389	0.286	0.392	0.328	0.181	-0.137	1.000

1=number of tillers, 2= height of main tiller, 3= peduncle length, 4=spike length, 5= spike width, 6=number of leaves, 7=flag leaf length, 8= flag leaf width, 9= 3rd leaf length, 10= 3rd leaf width, 11= stem girth, 12= number of nodes, 13= internode length.

Table 4.34: Variation for qualitative characters in pearl millet x *P. squamulatum* F_1 hybrids and BC_1 plants.

S.no	Character	Polymorphism	T1	T2	MA	H1	H2	H3	H4	H5	Polymorphism of the characters (%)					
											H1T1	T1H1	H1T2	T2H1	T1H2	T2H2
1	Node hairyness	Hairy (H)	H	H		H	H				95	85	100	100	100	100
		Less hairy (LH)						LH	LH	LH	5	15				
		Non hairy (NH)			NH											
2	Coloured ring at node	Yellow (Y)	Y		Y	Y						85	100	72.72		
		Violet (V)					V				70					30
		Light violet (LV)		LV				LV	LV	LV	30	15		27.27	25	40
		Dark violet (DV)													75	30
3	Leaf base colour	Yellow (Y)	Y	Y	Y	Y					60	70	33.3	45.45		
		Yellow + Violet (Y+V)					YV		YV		30	25	66.6	27.27	25	30
		Violet (V)						V			10	5		18.18		10
		Light violet(LV)								LV				9.09		
		Dark violet (DV)													75	60
4	Leaf Hairyness	Hairy (H)	H	H	H	H	H		H	H	50	35	33.3	45.45	100	100
		Less hairy (LH)						LH			50	65	66.6	54.54		
		Non hairy (NH)														
5	Leaf orientation	Straight (S)				S			S		10	15	33.3	18.18		10
		Semi drooping(SD)	SD	SD	SD		SD	SD			20	85	66.6	81.81		50
		Drooping (D)								D	70				100	40
6	Peripheral Sheath colour	Yellow (Y)	Y	Y	Y	Y			Y		75	90	100	81.81		
		Violet (V)					V	V		V	10	5			100	100
		Yellow + Violet (Y+V)									15	5		18.18		
7	Awn colour	Light violet(LV)								LV	10	10		18.18		10
		Yellow (Y)	Y	Y	Y	Y		Y	Y		60	75	100	54.54		
		Violet (V)									30	5		27.27	75	80
		Dark violet (DV)					DV					10			25	10
8	Stigma colour	White (W)	W	W		W					100	100	100	100	50	50
		Violet (V)			V		V	V	V	V					50	50
9	Stigma	Bifid (B)	B	B		B	B	B		B	100	95	100	100	100	100
		Bifid+Trifid (BT)			BT				BT			5				

T1= *P. glaucum* IG 99-748, T2= *P. glaucum* IG 2000-01, MA= *P. squamulatum* IG 98-360, H1, H2, H3, H4 and H5= F_1 hybrids between pearl millet and *P. squamulatum* H1T1, T1H1, T1H2 etc. denotes the BC_1 crosses between H1and T1, T1 and H1, T1and H2 respectively and so on.

Table 4.35: Cytological observations of pearl millet x *P. squamulatum* F_1 hybrids and BC_1 plants.

Plant	2n	PMC studied	Average chromosomal association at diakinesis								% chromosomes involved in bivalent formation	Chiasmata Frequency/ Range	Pollen stainability (%)
			I	II	III	IV	V	VI	VII	VIII			
P. glaucum IG 99-748 (= T1)	28	25	1.2	7.16	0.96	2.4					51.14	19.64	84
			(0-3)	(3-12)	(0-4)	(0-5)						(17-24)	
P. glaucum IG 2000-01 (= T2)	28	20	0.35	10.15	0.25	1.65					72.5	22.4	65
			(0-2)	(6-14)	(0-2)	(0-3)						(19-25)	
P. squamulatum IG 98-360 (= MA)	56	20		22.3	0.25	1.7	0.05	0.6			79.64	53.3	71
				(10-28)	(0-2)	(0-6)	(0-1)	(0-2)				(46-56)	
P. squamulatum IG 98-361 (= MB)	56	20	2.85	15.65	1.7	3.25	0.15	0.5			55.89	46.1	71
			(0-7)	(10-23)	(0-6)	(1-6)	(0-1)	(0-1)				(31-53)	
P. squamulatum IG 2000-36 (= MC)	56	20	0.3	19.8	1.5	2.9					70.71	44.4	74
			(0-2)	(16-26)	(0-4)	(1-6)						(39-51)	
F_1 Hybrids													
H1 (T1MA)	42	25	3.12	17.36	0.8	0.44					82.66	24.28	84
			(0-8)	(13-21)	(0-3)	(0-1)						(18-32)	
H2 (T1MA)	42	125	0.47	19.16	0.42	0.48					91.23	33.65	64
			(0-6)	(12-21)	(0-4)	(0-4)						(23-41)	
H3 (T1MC)	42	20	2	19.4	0.2	0.15					92.38	36.45	
			(0-6)	(14-21)	(0-2)	(0-2)						(29-42)	29
H4 (T2MC)	42	25	5.32	17.84	0.28	0.04					84.95	30.6	
			(0-10)	(14-21)	(0-2)	(0-1)						(25-34)	30
H5 (T2MC)	42	20		21							100.00	41.15	
												(34-42)	74
Avg of all (5) F_1 hybrids		215	1.41	18.99	0.39	0.35					90.42		
			(0-10)	(12-21)	(0-4)	(0-4)							
BC_1 plants													
H1T1	35	399	4.43	12.07	1.619	0.323	0.002	0.02			68.97		
			(0-15)	(3-17)	(0-7)	(0-4)	(0-1)	(0-2)					
H1T2	35	32	4.68	12.96	0.56	0.59	0.06				74.05		
			(1-7)	(10-17)	(0-4)	(0-2)	(0-1)						
T1H1	35	125	4.1	11.81	1.67	0.47	0.008	0.02	0.008	0.02	67.47		
			(0-13)	(6-17)	(0-6)	(0-3)	(0-1)	(0-1)	(0-1)	(0-1)			
T2H1	35	161	5.29	12.27	1.24	0.35		0.01			70.09		
			(0-25)	(4-17)	(0-7)	(0-2)		(0-1)					
T2H2	35	93	4.69	12.47	1.43	0.268					71.26		
			(0-10)	(6-17)	(0-7)	(0-2)							
T1H2	35	115	5.5	10.82	2.304	0.234					61.83		
			(0-16)	(5-17)	(0-7)	(0-2)							

*Figures in parenthesis denote range.

H1, H2, H3, H4 and H5= F_1 hybrids between pearl millet and *P. squamulatum*, T1MA= T1x MA, T1MC= T1xMC

H1T1, T1H1, T1H2 etc. denotes the BC_1 crosses between H1and T1, T1 and H1, T1and H2 respectively and so on.

Table 4.36: Fertility of BC_1 plants (pearl millet x *P. squamulatum*).

S. no.		Cross	Total no. of plants	Name
1.	Male and female fertile	H1T1	11	MP 5/5, MP 5/6, MP 5/7, 5 MP /11, MP 5/12, MP 5/14, MP 5/16, MP 5/17, MP 5/18, MP 5/19, MP 5/20
		H1T2	0	-
		T1H1	4	MP 5/29, MP 5/34, MP 5/35, MP 5/40
		T2H1	3	MP 4/4, MP 4/30, MP 4/31
		T1H2	0	-
		T2H2	2	MP 4/15, MP 4/34
2.	Male sterile and female fertile	H1T1	1	MP 5/8
		H1T2	1	MP 5/26
		T1H1	2	MP 5/32, MP 5/36
		T2H1	1	MP 4/5
		T1H2	0	-
		T2H2	0	-
3.	Self incompatible and female fertile	H1T1	8	MP 5/8, MP 5/10, MP 5/13, MP 5/15, MP 5/21, MP 5/22, MP 5/23, MP 5/24
		H1T2	2	MP 5/26, MP 5/27
		T1H1	4	MP 5/30, MP 5/36, MP 5/37, MP 5/39
		T2H1	4	MP 4/5, MP 4/6, MP 4/7, MP 4/8
		T1H2	0	-
		T2H2	1	MP 4/14

T1= *P. glaucum* IG 99-748, T2= *P. glaucum* IG 2000-01, H1 and H2, = F_1 hybrids between pearl millet (T1) and *P. squamulatum* (IG 98-360)
H1T1, T1H1, T1H2 etc. denotes the BC_1 crosses between H1and T1, T1 and H1, T1and H2 respectively and so on.

Table 4.37: Percentage of associations observed maximum times in pearl millet x *P. squamulatum* BC_1 generations.

S. no.	Cross →	H1T1	H1T2	T1H1	T2H1	T1H2	T2H2	H1T1 & H1T2	T1H1 & T2H1	T1H2 & T2H2
	Total no. of PMC studied→	399	32	125	161	115	93	431	286	208
	Total no. of chromosomes→	13965	1120	4375	5635	3255	4025	15085	10010	7280
	Associations↓									
1	$6_I + 10_{II} + 3_{III}$	2.25	0	2.4	1.24	0.008	0.02	2.08	1.74	1.44
2	$5_I + 15_{II}$	5.51	0	2.4	4.35	1.74	8.602	5.1	3.49	4.81
3	$4_I + 14_{II} + 1_{III}$	8.27	21.88	0	6.83	2.61	7.53	9.28	3.85	4.81
4	$7_I + 11_{II} + 2_{III}$	4.01	3.125	0	1.86	6.96	7.53	3.94	1.05	7.21
5	$3_I + 13_{II} + 2_{III}$	7.77	0	5.6	4.35	3.48	7.53	7.19	4.89	5.29
6	$5_I + 9_{II} + 4_{III}$	3.01	3.13	0	0.62	3.48	0	3.02	0.35	1.92
7	$7_I + 14_{II}$	3.01	31.25	2.4	3.11	0	5.38	5.1	2.79	2.4
8	$5_I + 12_{II} + 2_{III}$	7.27	0	9.6	4.35	3.48	8.6	6.73	6.64	5.77
9	$4_I + 11_{II} + 3_{III}$	2.76	2.86	4.8	0.62	6.96	3.23	2.78	2.45	5.29

T1= *P. glaucum* IG 99-748, T2= *P. glaucum* IG 2000-01, MA= *P. squamulatum* IG 98-360, H1 and H2, = F_1 hybrids between pearl millet and *P. squamulatum*
H1T1, T1H1, T1H2 etc. denotes the BC_1 crosses between H1and T1, T1 and H1, T1and H2 respectively and so on.

Table 4.38: Rm values of different isozymes in pearl millet x *P. squamulatum* F_1 hybrids and BC_1 plants.

Enzyme	E.C. No.	Band numbers																				
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Esterase	3.1.1.2	0.21	0.22	0.3	0.33	0.37	0.38	0.41	0.43	0.45	0.5	0.56	0.58	0.61	0.63	0.64	0.65	0.66	0.7	0.71	0.73	0.75
SOD	1.15.1.1	0.32	0.33	0.36	0.38	0.74	0.77	0.8														
POD	1.11.1.7	0.03	0.04	0.06	0.18	0.21																
Native protein		0.06	0.08	0.1	0.15	0.18	0.2	0.27	0.34	0.35	0.36	0.37	0.4	0.48	0.5	0.52	0.61					

Table 4.39: Banding pattern of pearl millet x *P. squamulatum* F_1 hybrids and BC_1 plants based on Esterase enzyme system.

S.no.	Band no.→	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
	RM →	0.21	0.22	0.3	0.33	0.37	0.38	0.41	0.43	0.45	0.5	0.56	0.58	0.61	0.63	0.64	0.65	0.66	0.7	0.71	0.73	0.75
	Plants↓																					
1	*P. squamulatum* IG 98-360	3+	3+			2+	2+				2+	2+							1+	3+	2+	1+
2	H1	1+	1+			1+	1+		1+	1+	1+	1+							2+	2+		
3	*P. glaucum* IG 99-748 (= T1)			1+	1+							3+	1+						3+	3+		
4	MP 5/24 (H1T1)	1+				2+	2+					3+							3+	3+		
5	MP 2/29 (H1T1)	1+				1+	1+					1+								2+		
6	MP 5/6 (H1T1)										3+	3+										
7	MP 5/8 (H1T1)											3+					2+		3+	3+		
8	MP 5/15 (H1T1)					2+	2+					3+							3+	3+		
9	MP 5/29 (T1H1)					2+						2+							3+	3+		
10	MP 5/31 (T1H1)	1+										2+							3+	3+		
11	MP 5/39 (T1H1)	1+										3+							3+	3+		
12	MP 5/32 (T1H1)	1+				2+	2+					3+							3+	3+		
13	MP 5/30 (T1H1)	1+										2+							3+	3+		
14	*P. glaucum* IG 2000-01 (= T2)			1+								2+		1+	1+		4+					
15	MP 5/26 (H1T2)	1+									2+	2+				2+			2+			
16	MP 2/18 (T2H1)						1+				2+	3+		2+		2+			3+			
17	MP 2/32 (T2H1)	1+		1+		1+					1+	3+							3+	2+	2+	
18	MP 4/30 (T2H1)											2+				2+	2+					
19	MP 4/7 (T2H1)					1+	1+				1+	3+		2+		2+			2+			
20	MP 4/3 (T2H1)					1+	1+				1+	2+		2+		2+	2+		2+			
21	MP 2/17 (T2H1)						1+				1+	3+		2+					2+			
22	MP 4/4 (T2H1)					1+	1+				1+	3+					3+		2+			
23	MP 4/31 (T2H1)					1+	1+				3+	3+						4+				
24	MP 4/6 (T2H1)											3+					3+	3+				
25	H2	2+	2+				3+				2+	3+							3+	3+	3+	
26	MP 4/15 (T2H2)	2+	2+				3+					3+		2+	3+			3+		2+		2+
27	MP 4/14 (T2H2)	3+	3+				2+				3+	3+				1+			2+		1+	2+
28	MP 4/12 (T2H2)						3+					2+			3+			2+	2+			
29	MP 2/24 (T2H2)						2+					3+						3+	3+			
30	MP 4/10 (T1H2)	2+					3+					3+		3+				2+	3+	2+		2+
31	MP 2/20 (T1H2)	1+	1+				3+					3+							2+	2+		2+
32	MP 2/21 (T1H2)	2+	2+				3+					3+		2+				1+	2+		2+	
33	MP 5/35 (T1H1)	2+						2+				3+							1+			3+

T1= *P. glaucum* IG 99-748, T2= *P. glaucum* IG 2000-01, H1 and H2, = F_1 hybrids between pearl millet (T1) and *P. squamulatum* (IG 98-360).
H1T1, T1H1, T1H2 etc. denotes the BC_1 crosses between H1and T1, T1 and H1, T1 and H2 respectively and so on.

Table 4.40: Banding pattern of pearl millet x *P. squamulatum* F_1 hybrids and BC_1 plants based on SOD enzyme system.

S. no.	Band no.→	1	2	3	4	5	6	7
	RM →	0.32	0.33	0.36	0.38	0.74	0.77	0.8
	Plants↓							
1	*P. squamulatum* IG 98-360	3+	3+	3+		3+	3+	3+
2	H1	3+	2+			3+	3+	2+
3	*P. glaucum* IG 99-748 (= T1)	3+	3+			3+		
4	MP 5/24 (H1T1)	3+	2+	3+	2+	3+	3+	3+
5	MP 2/29 (H1T1)	3+				3+		
6	MP 5/6 (H1T1)	3+				3+	3+	3+
7	MP 5/8 (H1T1)	3+				3+	3+	3+
8	MP 5/15 (H1T1)	3+	2+	3+		3+	3+	3+
9	MP 5/29 (T1H1)	3+	2+	3+		3+	3+	3+
10	MP 5/31 (T1H1)	3+	2+	3+		3+	3+	3+
11	MP 5/39 (T1H1)	3+			2+	3+	3+	3+
12	MP 5/32 (T1H1)	3+				3+	3+	3+
13	MP 5/30 (T1H1)	3+				3+	3+	3+
14	*P. glaucum* IG 2000-01 (= T2)	3+				3+	3+	3+
15	MP 5/26 (H1T2)	3+				3+	3+	3+
16	MP 2/18 (T2H1)	3+				3+	3+	3+
17	MP 2/32 (T2H1)	3+				3+	3+	3+
18	MP 4/30 (T2H1)	3+				3+	3+	3+
19	MP 4/7 (T2H1)	3+	3+			3+	3+	3+
20	MP 4/3 (T2H1)	3+	3+			3+	3+	3+
21	MP 2/17 (T2H1)	3+	3+			3+	3+	3+
22	MP 4/4 (T2H1)	3+	3+			3+	3+	3+
23	MP 4/31 (T2H1)	3+	3+			3+		
24	MP 4/6 (T2H1)	3+	3+			3+	3+	3+
25	H2	3+	3+	3+		3+	3+	3+
26	MP 4/15 (T2H2)	3+	3+	3+		3+	3+	
27	MP 4/14 (T2H2)	3+	3+	3+		3+	3+	3+
28	MP 4/12 (T2H2)	3+	3+	3+		3+		
29	MP 2/24 (T2H2)	3+	3+	3+		3+	3+	
30	MP 4/10 (T1H2)	3+	3+	3+		3+	3+	
31	MP 2/20 (T1H2)	3+	3+			3+	3+	3+
32	MP 2/21 (T1H2)	3+	3+			3+	3+	3+
33	MP 5/35 (T1H1)	3+	3+			3+	3+	

H1 and H2, = F_1 hybrids between pearl millet (T1) and *P. squamulatum* (IG 98-360).
H1T1, T1H1, T1H2 etc. denotes the BC_1 crosses between H1and T1, T1 and H1, T1 and H2 respectively and so on.

Table 4.41: Banding pattern of pearl millet x *P. squamulatum* F_1 hybrids and BC_1 plants based on Peroxidase enzyme system.

S. no.	Band no.→	1	2	3	4	5
	RM →	0.03	0.04	0.06	0.18	0.21
	Plants↓					
1	*P. squamulatum* IG 98-360	3+	2+	2+	3+	3+
2	H1	2+	2+	2+		3+
3	*P. glaucum* IG 99-748 (= T1)		1+	3+		3+
4	MP 5/24 (H1T1)	2+	3+			3+
5	MP 2/29 (H1T1)	2+	1+			3+
6	MP 5/6 (H1T1)	2+	2+			3+
7	MP 5/8 (H1T1)	2+	2+			3+
8	MP 5/15 (H1T1)	2+	2+			3+
9	MP 5/29 (T1H1)	2+	2+			3+
10	MP 5/31 (T1H1)	2+	2+			3+
11	MP 5/39 (T1H1)		2+			3+
12	MP 5/32 (T1H1)		2+			3+
13	MP 5/30 (T1H1)		2+			3+
14	*P. glaucum* IG 2000-01 (= T2)	2+	2+	2+		3+
15	MP 5/26 (H1T2)			2+		3+
16	MP 2/18 (T2H1)	2+		2+		3+
17	MP 2/32 (T2H1)	2+		2+		
18	MP 4/30 (T2H1)	2+	1+	2+		3+
19	MP 4/7 (T2H1)	2+		2+		3+
20	MP 4/3 (T2H1)			2+		3+
21	MP 2/17 (T2H1)			2+		3+
22	MP 4/4 (T2H1)			2+		3+
23	MP 4/31 (T2H1)			2+		3+
24	MP 4/6 (T2H1)		2+	2+		3+
25	H2	2+		2+		3+
26	MP 4/15 (T2H2)			2+		3+
27	MP 4/14 (T2H2)	2+		2+		3+
28	MP 4/12 (T2H2)			2+		3+
29	MP 2/24 (T2H2)	2+		2+		3+
30	MP 4/10 (T1H2)	2+		2+		3+
31	MP 2/20 (T1H2)	2+		2+		3+
32	MP 2/21 (T1H2)	2+		2+		3+
33	MP 5/35 (T1H1)	2+		2+		3+

H1 and H2, = F_1 hybrids between pearl millet (T1) and *P. squamulatum* (IG 98-360).
H1T1, T1H1, T1H2 etc. denotes the BC_1 crosses between H1and T1, T1 and H1, T1 and H2 respectively and so on.

Table 4.42: Banding pattern of pearl millet x *P. squamulatum* F_1 hybrids and BC_1 plants based on Native protein enzyme system.

S. no.	Band no.→	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	RM →		0.08	0.1	0.15	0.18	0.2	0.27	0.34	0.35	0.36	0.37	0.4	0.48	0.5	0.52	0.61
	Plants↓																
1	*P. squamulatum* IG 98-360		1+		1+	1+	1+	2+				1+		1+	2+	1+	1+
2	H1		1+		1+	1+		2+				1+		1+	2+		
3	*P. glaucum* IG 99-748 (= T1)		1+		1+	1+		2+		1+	1+	1+	1+	1+	2+	1+	
4	MP 5/24 (H1T1)		1+		1+	1+		2+				1+		1+	2+	1+	
5	MP 2/29 (H1T1)	1+	1+		1+			2+						1+	2+	1+	
6	MP 5/6 (H1T1)		1+					2+							2+	1+	
7	MP 5/8 (H1T1)		1+					2+	2+				1+	1+	2+	1+	
8	MP 5/15 (H1T1)				1+			2+			1+	1+		1+	2+	1+	
9	MP 5/29 (T1H1)					1+		2+			1+			1+	2+	1+	
10	MP 5/31 (T1H1)				1+	1+		2+	2+	1+	1+	1+		1+	2+	1+	
11	MP 5/39 (T1H1)				1+	1+		2+	2+	1+				1+	2+	1+	
12	MP 5/32 (T1H1)		1+		1+	1+		2+									
13	MP 5/30 (T1H1)		1+		1+			2+						1+	2+		
14	*P. glaucum* IG 2000-01 (= T2)		1+		1+	1+	1+	2+					1+	1+	2+	1+	
15	MP 5/26 (H1T2)					1+	1+	2+							2+	1+	
16	MP 2/18 (T2H1)					1+	1+	2+							2+		
17	MP 2/32 (T2H1)					1+	1+	2+							2+	1+	
18	MP 4/30 (T2H1)					1+	1+	2+							2+	1+	
19	MP 4/7 (T2H1)				1+	1+	1+	2+							2+	1+	
20	MP 4/3 (T2H1)				1+	1+	1+	2+					1+		2+	1+	
21	MP 2/17 (T2H1)		1+		1+	1+	1+	2+					1+		2+	1+	
22	MP 4/4 (T2H1)		1+			1+	1+	2+					1+		2+	1+	
23	MP 4/31 (T2H1)		1+		1+	1+	1+	2+					1+				
24	MP 4/6 (T2H1)					1+	1+	2+				1+	1+	1+	2+	1+	
25	H2			1+	1+		1+	2+			1+				2+		
26	MP 4/15 (T2H2)					1+		2+			1+	1+			2+		
27	MP 4/14 (T2H2)							2+			1+	1+			2+	1+	1+
28	MP 4/12 (T2H2)					1+	1+	2+				1+			2+		
29	MP 2/24 (T2H2)							2+			1+	1+	1+	1+	2+		
30	MP 4/10 (T1H2)							2+				1+	1+	1+	2+		
31	MP 2/20 (T1H2)					1+		2+			1+	1+	1+	1+	2+		
32	MP 2/21 (T1H2)					1+	1+	2+				1+	1+	1+	2+		
33	MP 5/35 (T1H1)					1+		2+			1+						

H1 and H2, = F_1 hybrids between pearl millet (T1) and *P. squamulatum* (IG 98-360).

H1T1, T1H1, T1H2 etc. denotes the BC_1 crosses between H1and T1, T1 and H1, T1 and H2 respectively and so on.

Table 4.43: Morphological observations (qualitative) of GO & GOS hybrids.

S.no.	Plant identity	Cross	node hairiness	node colour	leaf base		leaf sheath hairiness	leaf		Spike (awn) colour	stigma	
					colour	hairiness		drooping/ straight	colour		colour	bifid/ trifid
1	MP 3/6	F_1(GO)	LH	Y	Y	NH	NH	S	G	Y+V	W	B
2	Plant 1	BC_1 (GO)	H	Y	Y+V	NH	NH	SD	G	Y+V	W	B
3	Plant 2	BC_1 (GO)	H	Y	Y+V	NH	NH	SD	G	Y+V	W +V	B
4	Plant 3	BC_1 (GO)	H	Y	Y+V	NH	NH	SD	G	Y+V	W	B
5	*P. orientale*	IG 04-165	H	LV	Y+V	H	H	SD	LG	V	DV	B
6	***P. glaucum***	81A1	NH	V	LV	H	H	SD	G	Y	W	B
7	***P. glaucum***	81A4	NH	V	LV	H	H	SD	G	Y	W	B
8	***P. glaucum***	IG 99-748 (=T1)	H	Y	Y	H	H	SD	G	Y	W	B
9	***P. glaucum***	IG 2000-01 (=T2)	H	LV	Y	H	H	SD	G	Y	W	B
10	*P. squamulatum*	IG 98-360	NH	Y	Y	H	NH	SD	DG	Y	DV	B+T
11	H1	T1 x IG 98-360	H	Y	Y+V	H	H	SD	DG	Y	W	B+T
12	H2	T1 x IG 98-360	H	V	Y+V	H	H	SD	DG	V	V	B
13	Hybrid 1	BC_1 (GO) x T1	H	G	Y	NH	NH	SD	DG	Y	W	B
14	Hybrid 2	BC_1 (GO) x T2	H	G	Y	LH	NH	SD	DG	Y	W	B
15	Hybrid 3	BC_1 (GO) x T2	H	G	Y	H	H	SD	DG	Y	W	B
16	Hybrid 4	BC_1 (GO) x H1	LH	G	Y	H	NH	S	G	Y	W	B+T
17	Hybrid 5	BC_1 (GO) x H2	H	G	Y	NH	NH	SD	DG	Y+V	V	B
18	Hybrid 6	BC_1 (GO) x H2	H	G	Y	NH	NH	SD	DG	Y+V	W	B
19	Hybrid 7	BC_1 (GO) x H2	H	G	Y	NH	NH	SD	DG	Y	W	B
20	Hybrid 8	BC_1 (GO) x H2	NH	Y	Y	NH	NH	SD	DG	Y	W	B
21	Hybrid 9	BC_1 (GO) x H2	NH	Y	Y	NH	NH	SD	DG	Y	W	B
22	Hybrid 10	81A4 X H2	H	Y	Y	H	NH	SD	G	Y+V	W	B

F_1 (GO) = F_1 hybrid between diploid *P. glaucum* and diploid *P. orientale*, BC_1 (GO) = BC_1 of F_1 (GO) and diploid *P. glaucum*, T1 = *P. glaucum* IG 99-748, T2 = *P. glaucum* IG 2000-01, H1 and H2 = F_1 hybrid between *P. glaucum* IG 99-748 (= T1) and *P. squamulatum* IG 98-360.

LH-less hairy, H-hairy, NH-non hairy, LG- light green, G-green, DG-dark green, Y-yellow, W-white, V-violet, LV-light violet, DV- dark violet, D- drooping, SD-semi drooping, S-straight, B-bifid, T-trifid. Y+V= yellow and violet both colours observed in the same plant, B+T= bifid and trifid both stigma found in the same spike.

Table 4.44: Morphological observations (quantitative) of GO & GOS hybrids.

S.no.	Plant identity	Cross/accession no.	No. of tillers	Height of maintiller	No. of leaves	Flag leaf		3rd leaf		Stem girth (cm)	No. of nodes	Internode length (cm)	Peduncle length (cm)	spike		No. of florets/ spikelet
						length (cm)	width(cm)	length (cm)	width (cm)					length (cm)	width (cm)	
1	MP 3/6	F_1(GO)	55	102	7	18	1	29	1.1	0.3	7	11	30	11.5	1.2	1-4
2	Plant 1	BC_1 (GO)	25	65	6	15	0.6	27	1.1	0.2	5	10	22	3	1	2-3
3	Plant 2	BC_1 (GO)	38	33	7	13	0.9	29	1.1	0.3	6	13	25	10	1.5	1-2
4	Plant 3	BC_1 (GO)	38	81	6	9	0.5	21	1.1	0.3	6	14	23	13	1.4	1-2
5	*P. orientale*	IG 04-165	15	61	14.67	21.67	0.43	28.67	0.43	0.27	9.67	5	21.67	13.67	1.17	2-3
6	*P. glaucum*	81A1	8	110	12	26	2.5	40	3	1	10	13	30	26	3	3
7	*P. glaucum*	81A4	4	72	10	15	1	29	2	1	6	12	20	25	1.5	2-3
8	*P. glaucum*	IG 99-748 (= T1)	3.33	136.6	6.33	42.66	3.5	71.66	3.66	1.66	6	15.33	38.3	25.66	3.33	4
9	*P. glaucum*	IG 2000-01(= T2)	2	100	8	27	3	48	4	1.29	3	17	22	20	3	4
10	*P. squamulatum*	IG 98-360	20	186.33	10.67	31.33	1.4	68.67	1.6	0.57	10.67	16	58	31.5	2	14
11	H1	T1 x IG 98-360	3	80	6	16.67	0.9	43.17	1.03	0.5	5.3	11.67	30.67	19	1.6	3-8
12	H2	T1 x IG 98-360	15.3	104	8.67	22.17	1.07	44.3	1.23	0.63	7	7.83	30.33	26.33	1.93	4-10
13	Hybrid 1	BC_1 (GO) x T1	4	35	6	24	1.5	22	1.5	0.5	5	10	20	12	1.5	2-4
14	Hybrid 2	BC_1 (GO) x T2	4	136	6	27	3.5	49	3.8	1	7	19	30	15	2	2-6
15	Hybrid 3	BC_1 (GO) x T2	5	80	6			33	1.8	0.5	6	13.5	22	12	2	2-3
16	Hybrid 4	BC_1 (GO) x H1	5	129	9	9	1.5	42	2	0.8	7	16	36	15	1.5	2-6
17	Hybrid 5	BC_1 (GO) x H2	5	92	8	21	1.2	48	1.3	0.5	6	11	21	16	2	2-5
18	Hybrid 6	BC_1 (GO) x H2	25	87	8	22	1.1	41.5	1.3	0.3	6	10	23	16	1.7	2-3
19	Hybrid 7	BC_1 (GO) x H2	7	139	10	11	0.6	26	1.7	0.7	9	16	26	10	2.2	1-3
20	Hybrid 8	BC_1 (GO) x H2	43	40	6	5	0.4	10	0.5	0.2	5	4	22	12	1	1-2
21	Hybrid 9	BC_1 (GO) x H2	24	35	6	8	0.8	1-2	4	4	0.4	15	0.6	0.4	3	3
22	Hybrid 10	81A4 x H2	17	130	14	14	0.6	31	1.1	0.7	12	13	31	21	2.5	3-6

F_1 (GO) = F_1 hybrid between diploid *P. glaucum* and diploid *P. orientale*, BC_1 (GO) = BC_1 of F_1 (GO) and diploid *P. glaucum*, T1 = *P. glaucum* IG 99-748, T2 = *P. glaucum* IG 2000-01, H1 and H2 = F_1 hybrid between *P. glaucum* IG 99-748 (= T1) and *P. squamulatum* IG 98-360.

Table 4.45: Cytological observations of GO and GOS hybrids.

S.no.	Plant identity	Cross/accession	2n*	Average chromosomal configuration and range+ at diakinesis							Chiasmata % & range*	Pollen stainability %
				PMC studied	I	II	III	IV	V	VI		
1	MP 3/6	F_1(GO)	16 (7G+9O)	15	16							0%
2	Plant 2	BC_1(GO)	(14G+9O)	25	0.16(0-2)G	6.92(6-7)G						0%
					3.56(1-7)O	2.72(1-4)O						
3	Plant 3	BC_1(GO)	(14G+9O)	25	0.24(0-2)G	6.88(6-7)G					13.56	0%
					7.4(7-9)O	0.8(0-1)O					(11-15)	
4	Hybrid 1	BC_1(GO) x T1	37	25	7.68	10.4	0.12	2.04			24.12	0%
			(28G+9O)		(7-11)	(6-15)	(0-3)	(0-4)			(20-28)	
5	Hybrid 2	BC_1(GO) x T2	37	25	6.88	12.92	0.28	0.8		0.04	24.56	0%
			(28G+9O)		(2-12)	(9-16)	(0-3)	(0-3)		(0-1)	(20-28)	
6	Hybrid 3	BC_1(GO) x T2	37	25	7.2	10.44	1.16	1.36			22.8	2
			(28G+9O)		(4-9)	(6-13)	(0-3)	(0-3)			(19-26)	
7	Hybrid 4	BC_1(GO) x H1	44	25	10.24	13.88	2				20.6	0
			(21G+14S+9O)		(5-16)	(10-18)	(0-4)				(14-28)	
8	Hybrid 5	BC_1(GO) x H2	44	50	18.56	12.6	0.08				12.76	33.53
			(21G+14S+9O)		(10-28)	(8-17)	(0-1)				(8-17)	
9	Hybrid 6	BC_1(GO) x H2	44	25	7.2	15.04	2.16	0.04			24.08	0
			(21G+14S+9O)		(2-14)	(12-21)	(0-4)	(0-1)			(19-29)	
10	Hybrid 7	BC_1(GO) x H2	44	18	4.28	15	1.36	1.39			27.39	2
			(21G+14S+9O)		(0-10)	(10-22)	(0-3)	(0-4)			(23-33)	
11	Hybrid 8	BC_1(GO) x H2	44	25	3.32	13.6	2.28	1.68			28.56	0
			(21G+14S+9O)		(1-8)	(9-20)	(0-5)	(0-4)			(22-33)	
12	Hybrid 10	81A1 X H2	35	25	3	11.4	3.08				22	0
			(21G+14S)		(0-6)	(7-17)	(0-5)				(18-26)	
13	***P. glaucum***	81A1	14	30		7					13.9	
											(11-16)	
14	***P. orientale***	IG 04-165	36		0.13	22.6		2.1				85.09
					(0-2)	(19-26)		(0-4)				
15	***P. glaucum***	IG 99-748 (= T1)	28		1.2	7.16	0.96	2.4			19.64	84.58
					(0-3)	(3-12)	(0-4)	(0-5)			(17-24)	
16	***P. glaucum***	IG 2000-01(= T2)	28		0.35	10.15	0.3	1.7			22.4	65
					(0-2)	(6-14)	(0-2)	(0-3)			(19-25)	
17	*P. squamulatum*	IG 98-360	56	20		22.3	0.25	1.7	0.05	0.6	53.3	71
						(10-28)	(0-2)	(0-6)	(0-1)	(0-2)	(46-56)	
18	H1	T1 x IG 98-360	42	25	3.12	17.36	0.8	0.44			24.28	84
					(0-8)	(13-21	(0-3)	(0-1)			(18-32)	
19	H2	T1 x IG 98-360	42	125	0.47	19.16	0.42	0.48				64
					(0-6)	(12-21)	(0-4)	(0-4)				

*G= glaucum chromosomes, O= orientale chromosomes, +Figures in parenthesis denote range.

F_1 (GO) = F_1 hybrid between diploid *P. glaucum* and diploid *P. orientale*, BC_1 (GO) = BC_1 of F_1 (GO) and diploid *P. glaucum*, T1 = *P. glaucum* IG 99-748, T2 = *P. glaucum* IG 2000-01, H1 and H2 = F_1 hybrid between *P. glaucum* IG 99-748 (or T1) and *P. squamulatum* IG 98-360

LATE 1: MORPHOLOGY OF *PENNISETUM* SPECIES
ıtra- and inter-specific variation in *Pennisetum* species (from left to right)
ɔikes
P. glaucum (IG 99-748 or T1)
P. squamulatum (IG 98-360, IG 98-361, IG 2000-36)
P. ramosum (IP 22180, IP 22137, IP 21935)
P. orientale (IP 22186, IP 21951, IG 04-165, IG 04-165)
P. polystachyon (IP 22102, IP 22109, IP 22121, IP 21904, IP 21900)
P. violaceum (IP 21634), *P. mollisimum* (21782), *P. glaucum* (81B), *P. schweinfurthii* (IP 21931).
P. pedicellatum (Agros 4), *P. orientale* (IG 04-165), *P. squamulatum* (IG 2000-36).
P. setaceum (IP 21949).
des
P. pedicellatum (NATP D-1, IP 22095, IP 21890, IP 21883)
P.glaucum (81B), *P. orientale* (IG 04-165), *P. pedicellatum* (Agros 4).
P. violaceum (IP 21634), *P. mollisimum* (IP 21782), *P. glaucum* (81B), *P. schweinfurthii* (IP 21931).
P. polystachyon (IP 22102, IP 22109, IP 22121, IP 21904, IP 21900)
kelet
P. ramosum (IP 22180), *P. mollisimum* (IP 21782), *P. violaceum* (IP 21634), *P. ıcum* (81B), *P. schweinfurthii* (IP 21931).
P. pedicellatum (IP 22095), *P. polystachyon* (IP 22102, IP 22121), *P. orientale* (IG)4-165), *P. squamulatum* (IG 2000-36).
P. setaceum (IP 21949), *P. pedicellatum* (Agros 4)
P. squamulatum (IG 2000-36), *P. glaucum* (T1).
ls
P. glaucum (81B, 81A1, T1), *P. violaceum* (IP 21579), *P. mollisimum* (IP 21782), *P. ıceum* (IP 21532).
P. ramosum (IP 22137), *P. pedicellatum* (IP 22095, IP 21971, NATP D-1), *P. olystachyon* (IP 22109), *P. villosum* (IP 21945).
P. flassidum (IP 22195, IP 22188), *P. setaceum* (IP 21949, IP 22169), *P. hoheneckeri* IP 21954, IP 21953).
P. divisum (IP 21962, IP 22142, IP 21957), *P. orientale* (IP 22186), *P. setosum* (IP 1942), *P. schweinfurthii* (IP 21929).

PLATE 1: MORPHOLOGY OF *PENNISETUM* SPECIES

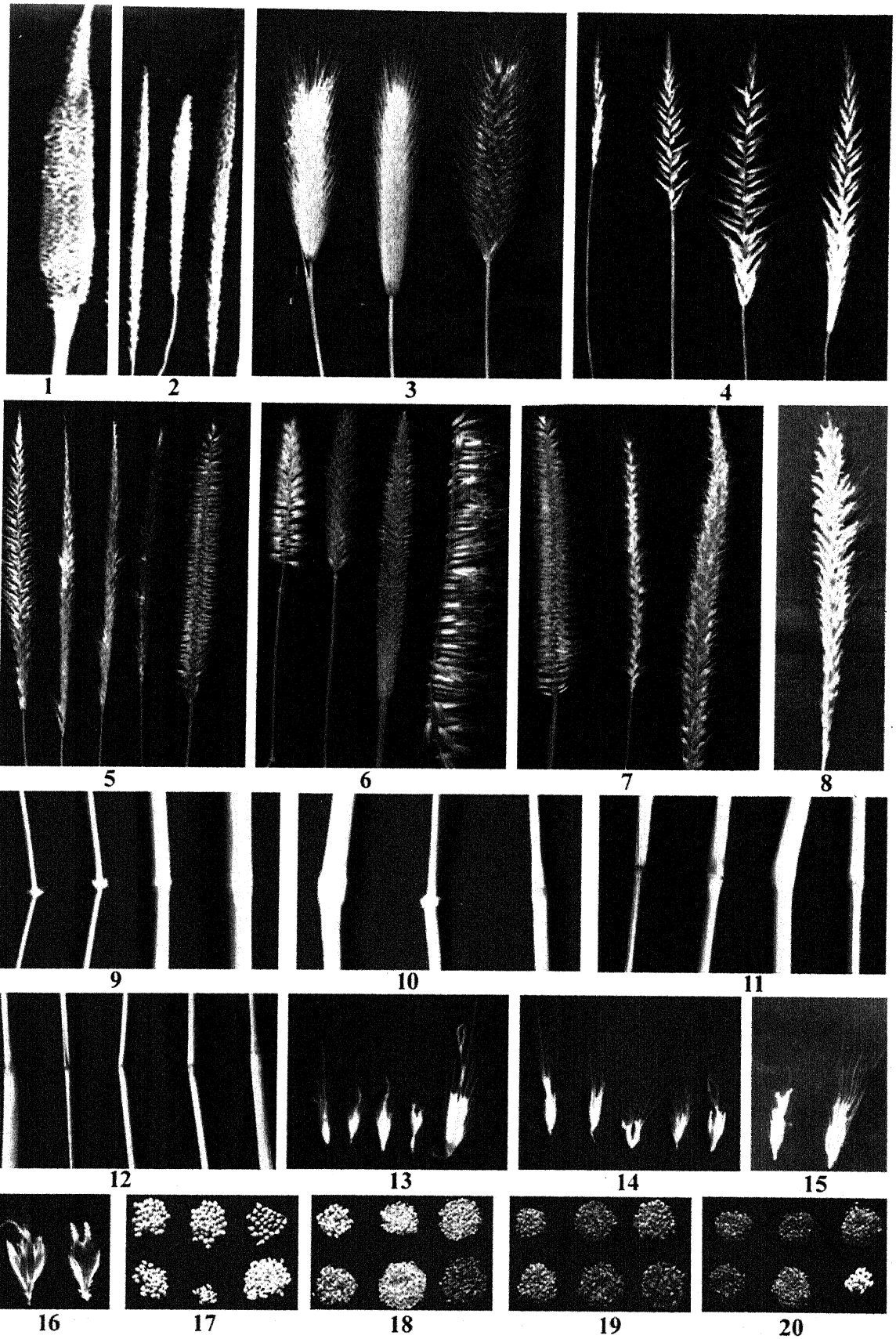
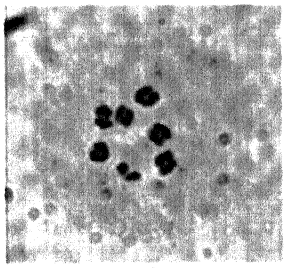


PLATE 2: MEIOSIS IN *PENNISETUM* SPECIES

1. *P. glaucum* (81B) diakinesis (7_{II})
2. *P. glaucum* (81B) pollen
3. *P. glaucum* (T1) diakinesis ($4_I + 4_{II} + 4_{IV}$)
4. *P. glaucum* (T1) anaphase I (14+14)
5. *P. glaucum* (T1) pollen
6-7. *P. violaceum* (PV 2433) diakinesis (7_{II})
8. *P. violaceum* (PV 2433) pollen
9. *P. mollisimum* (IP 21782) diakinesis (7_{II})
10. *P. mollisimum* (IP 21782) pollen
11. *P. schweinfurthii* (PS 233) diakinesis (7_{II} + 2 Bs)
12. *P. schweinfurthii* (PS 233) pollen
13. *P. ramosum* (IP 22180) diakinesis (5_{II})
14. *P. ramosum* (IP 22180) pollen
15. *P. orientale* (IG 04-165) diakinesis ($25_{II} + 1_{IV}$)
16. *P. orientale* (IG 04-165) pollen
17. *P. divisum* (IP 21962) diakinesis ($6_I + 8_{II} + 3_{IV}$)
18. *P. divisum* (IP 21962) pollen
19. *P. setaceum* (IP 21949) diakinesis ($9_I + 9_{II}$)
20. *P. setaceum* (IP 21949) pollen.

PLATE 2: MEIOSIS IN *PENNISETUM* SPECIES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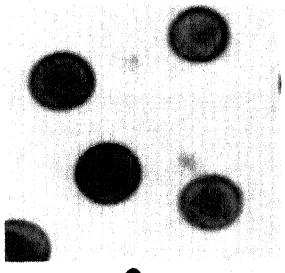
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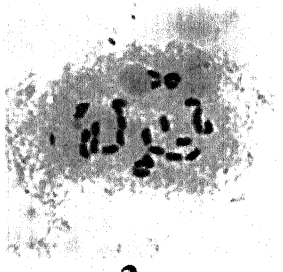
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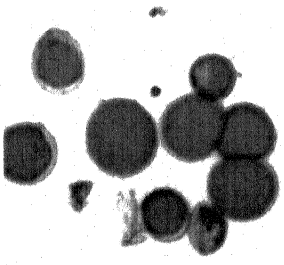
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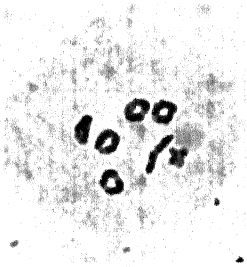
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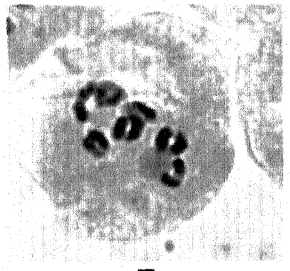
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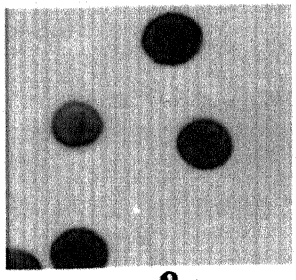
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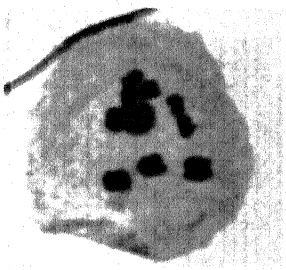
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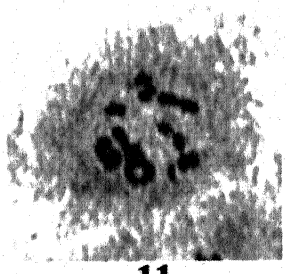
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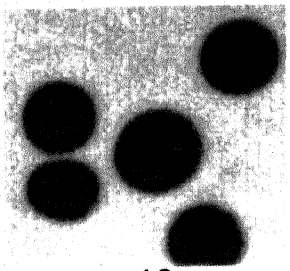
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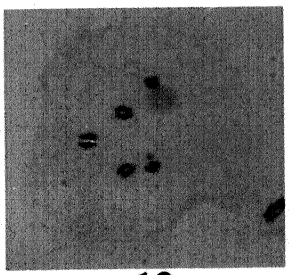
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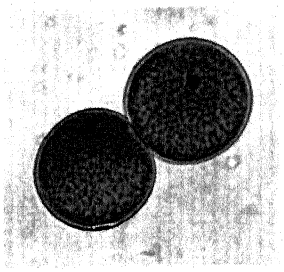
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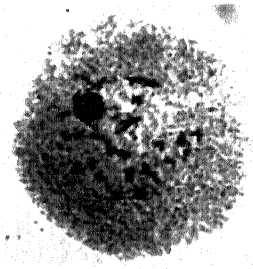
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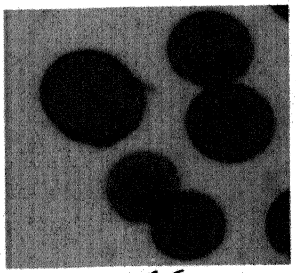
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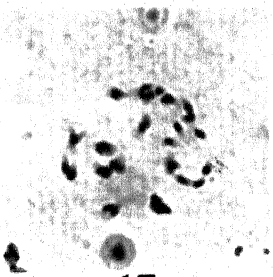
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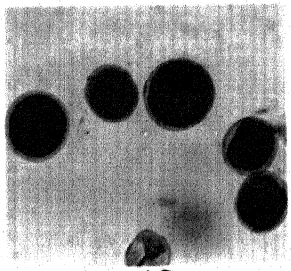
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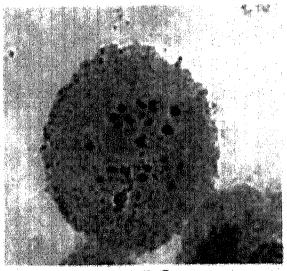
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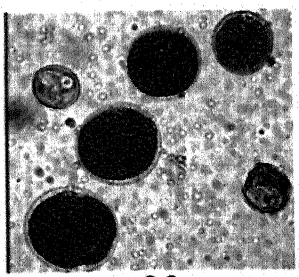
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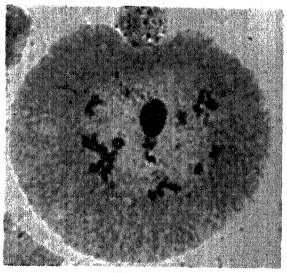
PLATE 2: (Contd.)

21. *P. flassidum* (IP 22195) diakinesis ($1_I + 20_{II} + 1_{IV}$)
22. *P. flassidum* (IP 22195) Anaphase I (lagging chromosomes)
23. *P. flassidum* (IP 22195) pollen
24. *P. hoheneckeri* (IP 21954) diakinesis (9_{II})
25. *P. hoheneckeri* (IP 21954) dyad (9+9)
26. *P. hoheneckeri* (IP 21954) anaphase I
27. *P. polystachyon* (IP 22102) diakinesis ($22_{II} + 2_{III} + 1_{IV}$)
28. *P. polystachyon* (IP 22102) anaphase I (2n=54)
29. *P. polystachyon* (IP 22102) pollen
30. *P. pedicellatum* (IP 21879) anaphase I (2n=54)
31. *P. pedicellatum* (IP 21879) pollen
32. *P. pedicellatum* (IP 22095) diakinesis ($15_{II} + 2_{III}$)
33. *P. pedicellatum* (IP 22095) pollen
34. *P. pedicellatum* (Agros 4) diakinesis ($2_I + 19_{II} + 4_{III} + 5_{IV}$)
35. *P. pedicellatum* (Agros 4) anaphase I (2n=72)
36. *P. pedicellatum* (Agros 4) pollen
37. *P. squamulatum* (IG 98-360) diakinesis ($15_{II} + 2_{III} + 5_{IV}$)
38. *P. squamulatum* (IG 98-360) anaphase I (2n=56)
39. *P. squamulatum* (IG 2000-36) diakinesis ($26_{II} + 1_{IV}$)
40. *P. squamulatum* (IG 2000-36) pollen

PLATE 2: (Contd.)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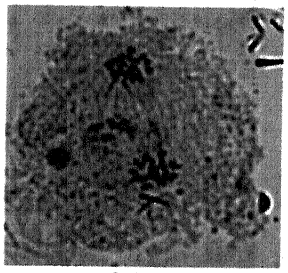
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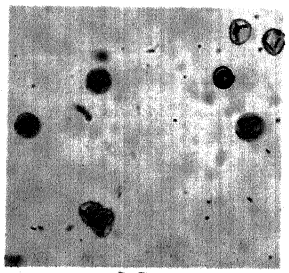
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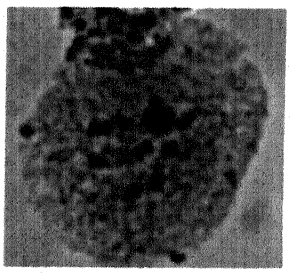
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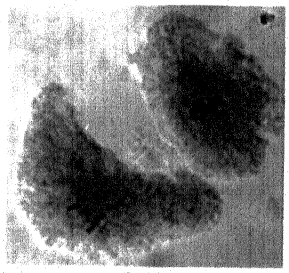
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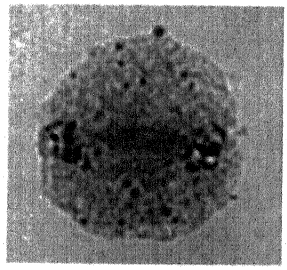
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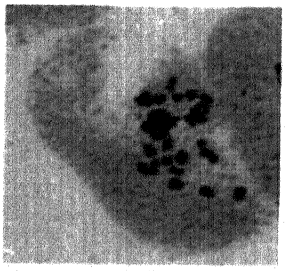
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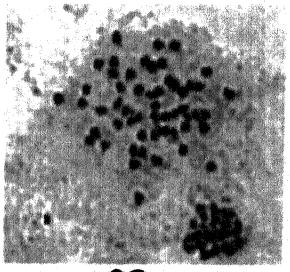
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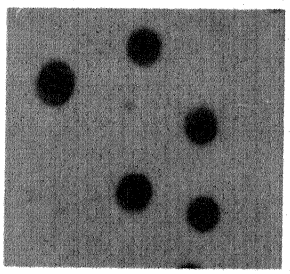
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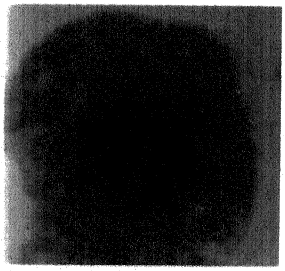
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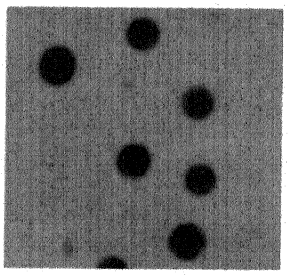
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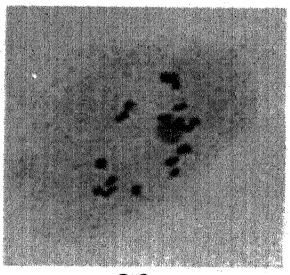
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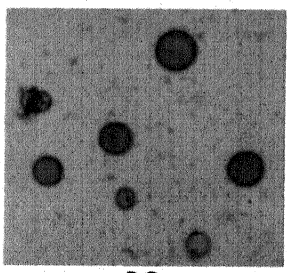
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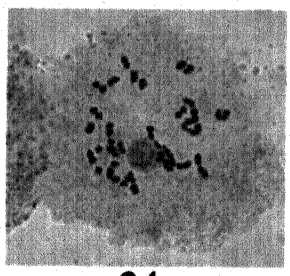
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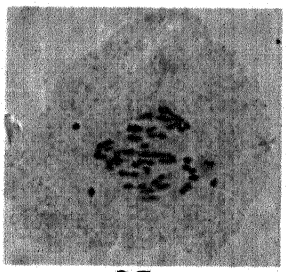
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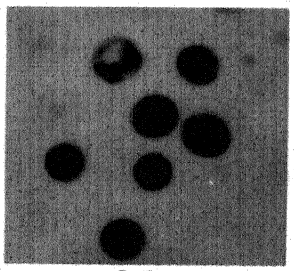
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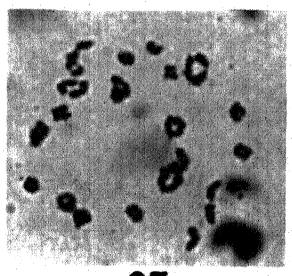
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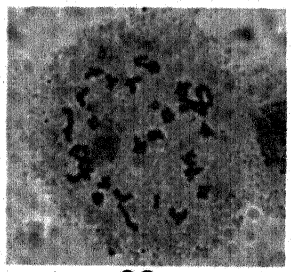
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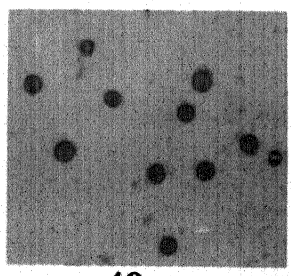
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PLATE 3: ISOZYMES IN *PENNISETUM* SPECIES

1. Esterase set 1 (PAGE) (samples from left to right)
P. squamulatum IG 98-360, *P. orientale* IP 21951, IP 22186, IG 04-165, *P. setaceum* IP 21949, *P. polystachyon* IP 22102, IP 22109, IP 22121, *P. divisum* IP 21957, IP 21962, *P. villosum* IP 21945, *P. squamulatum* IG 2000-36, *P. squamulatum* IG 98-361.

2. Esterase set 2 (PAGE) (samples from left to right)
P. squamulatum IG 98-360, *P. flassidum* IP 22195, IP 22200, IP 22188, *P. hoheneckeri* IP 21954, IP 21953, IP 21952, *P. setosum* IP 21942, *P. clendenstenum* IG 04-166, *P. pedicellatum* NATP D-1, IP 21971 (6x), IP 22095 (4x), *P. squamulatum* IG 98-360.

3. Esterase set 3 (PAGE) (samples from left to right)
P. squamulatum IG 98-360, *P. orientale* IP 21951, IP 22186, IG 04-165, *P. setaceum* IP 21949, *P. polystachyon* IP 22102, IP 22109, IP 22121, *P. divisum* IP 21957, IP 21962, *P. villosum* IP 21945, *P. squamulatum* IG 2000-36, *P. squamulatum* IG 98-361.

4. Superoxide Desmutase set 1 (PAGE) (samples from left to right)
P. squamulatum IG 98-360, *P. orientale* IP 21951, IP 22186, IG 04-165, *P. setaceum* IP 21949, *P. polystachyon* IP 22102, IP 22109, IP 22121, *P. divisum* IP 21957, IP 21962, *P. villosum* IP 21945, *P. squamulatum* IG 2000-36, *P. squamulatum* IG 98-361.

5. Superoxide Desmutase set 2 (PAGE) (samples from left to right)
P. squamulatum IG 98-360, *P. flassidum* IP 22195, IP 22200, IP 22188, *P. hoheneckeri* IP 21954, IP 21953, IP 21952, *P. setosum* IP 21942, *P. clendenstenum* IG 04-166, *P. pedicellatum* NATP D-1, IP 21971 (6x), IP 22095 (4x), *P. squamulatum* IG 98-361.

6. Superoxide Desmutase set 3 (PAGE) (samples from left to right)
P. squamulatum IG 98-360, *P. orientale* IP 21951, IP 22186, IG 04-165, *P. setaceum* IP 21949, *P. polystachyon* IP 22102, IP 22109, IP 22121, *P. divisum* IP 21957, IP 21962, *P. villosum* IP 21945, *P. squamulatum* IG 2000-36, *P. squamulatum* IG 98-361.

7. Peroxidase set 1 (starch gel) (samples from left to right)
P. glaucum T1, T2 (or IG 2000-01), 81B, 81A1, *P. violaceum* PV 2433, IP 21634, IP 21532, IP 21524, IP 21634, IP 21579, *P. squamulatum* IG 98-360.

8. Peroxidase set 2 (starch gel) (samples from left to right)
P. squamulatum IG 98-360, unidentified species, *P. schweinfurthii* PS 2116, IP 21931, IP 21929, PS 237, *P. mollisimum* IP 21782, *P. ramosum* IP 22180, IP 21935, IP 22137.

9. Peroxidase set 3 (starch gel) (samples from left to right)
P. squamulatum IG 2000-36, *P. squamulatum* IG 98-361, *P. divisum* IP 21962, *P. flassidum* IP 22195, *P. squamulatum* IG 98-360, *P. squamulatum* IG 2000-36, *P. setaceum* IP 21949, IP 21949 IPK, *P. orientale* IG 04-165, IP 21951.

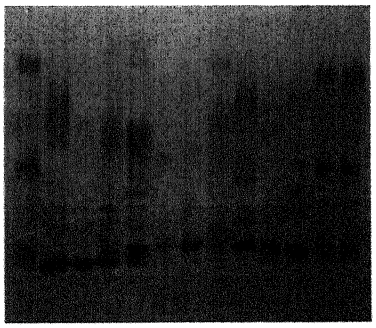
10. GDH set 1 (PAGE) (samples from left to right)
P. glaucum T1, T2, *P. mollisimum* IP 21782, *P. violaceum* IP 21532, IP 21634, *P. squamulatum* IG 98-360.

11. GDH set 2 (PAGE) (samples from left to right)
P. clendenstenum IG 04-166, *P. pedicellatum* NATP D-1, *P. polystachyon* IP 22102, IP 21971, IP 22095.

12. GDH set 3 (PAGE) (samples from left to right)
P. squamulatum IG 98-360, *P. flassidum* IP 22195, IP 22188.

13. GDH set 3 (PAGE) (samples from left to right)
P. squamulatum IG 98-360, *P. setaceum* IP 21949, *P. orientale* IP 21951, IP 22186, *P. setosum* IP 21942.

PLATE 3: ISOZYMES IN *PENNISETUM* SPECIES



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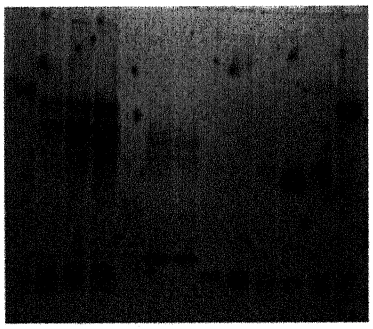

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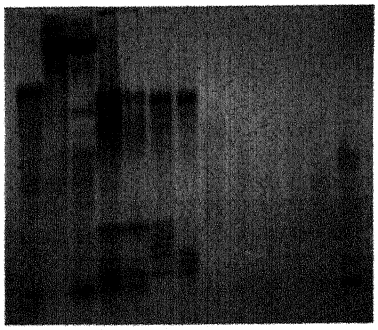

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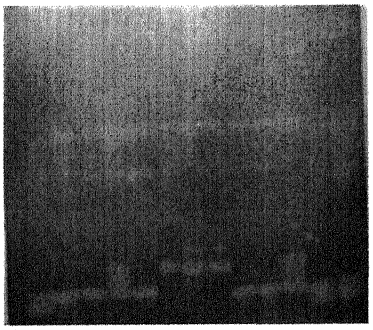

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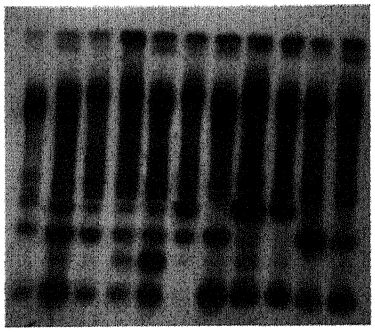

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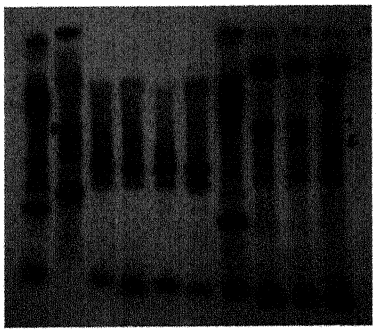

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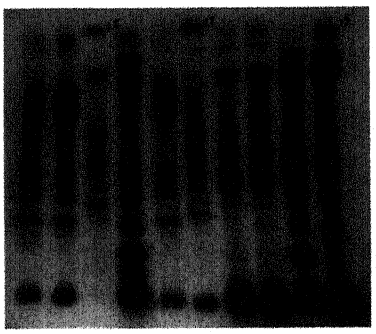

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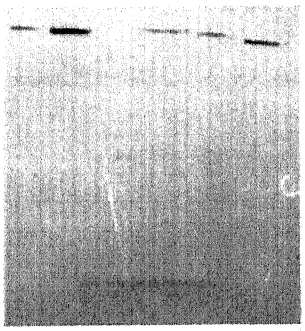

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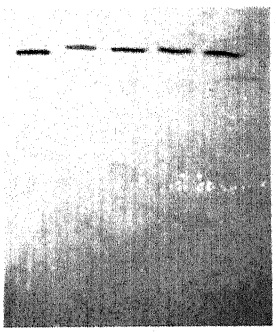

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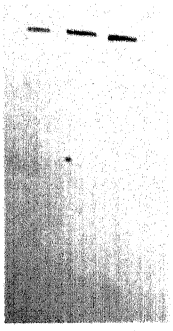

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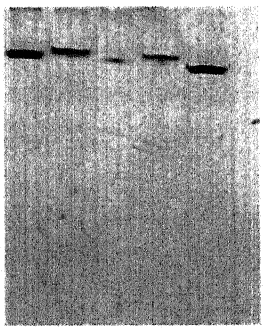

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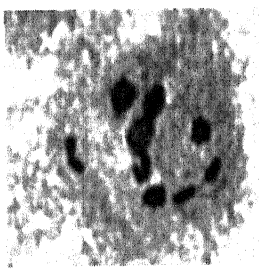
PLATE 4: DIPLOID AND INDUCED TETRAPLOID *P. SCHWEINFURTHII*

1-2. *P. schweinfurthii* (PS 233 diploid) diakinesis 7_{II}
3. *P. schweinfurthii* (2x) metaphase I
4. *P. schweinfurthii* (2x) anaphase I
5. *P. schweinfurthii* (2x) pollen (magnification 10x)
6. *P. schweinfurthii* (2x) pollen (magnification 40x)
7-9. *P. schweinfurthii* (induced tetraploid) diakinesis 2n=28
10. *P. schweinfurthii* (induced tetraploid) metaphase I
11. *P. schweinfurthii* (induced tetraploid) metaphase I with two univalents
12-13. *P. schweinfurthii* (induced tetraploid) anaphase I 2n=28
14. *P. schweinfurthii* (induced tetraploid) telophase I
15. *P. schweinfurthii* (induced tetraploid) pollen (magnification 10x)
16. *P. schweinfurthii* (induced tetraploid) pollen (magnification 40x)
17-18. *P. schweinfurthii* (induced tetraploid) seeds open pollinated.
19-20. *P. schweinfurthii* (induced tetraploid) seeds self-pollinated.

PLATE 4: DIPLOID AND INDUCED TETRAPLOID *P. SCHWEINFURTHII*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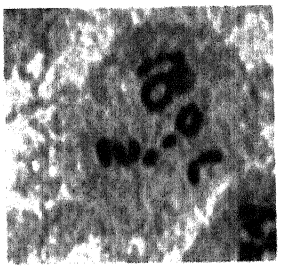
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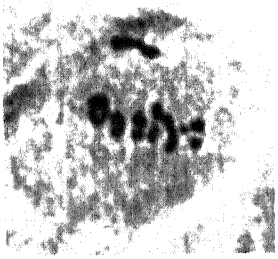
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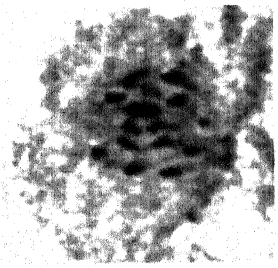
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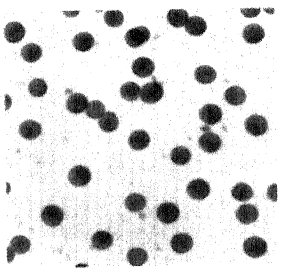
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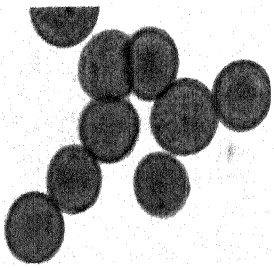
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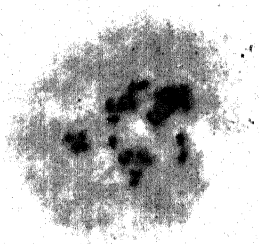
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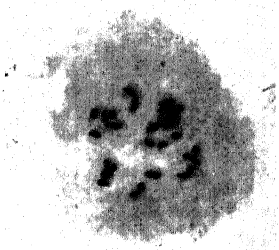
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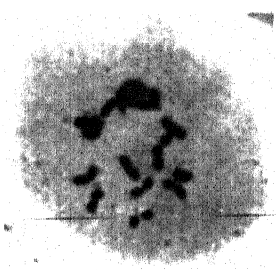
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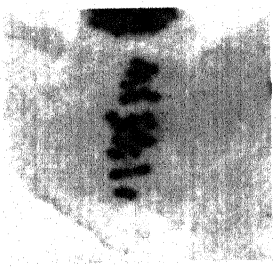
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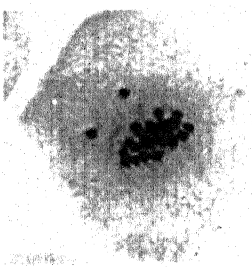
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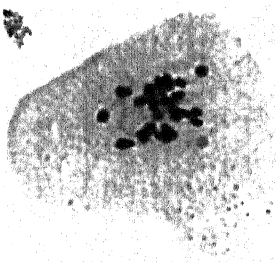
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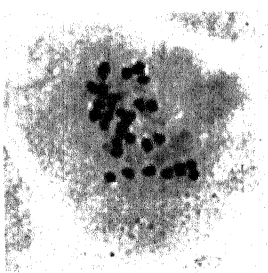
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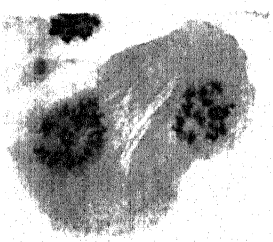
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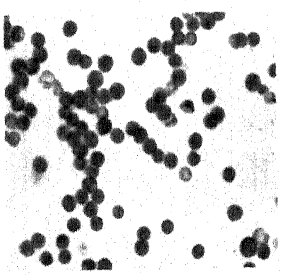
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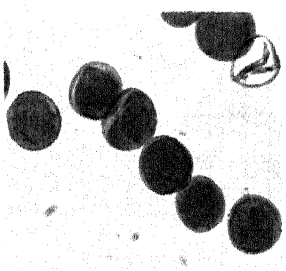
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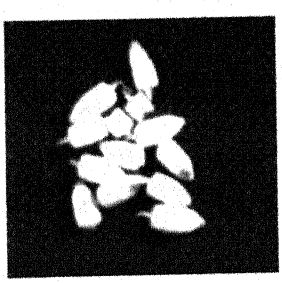
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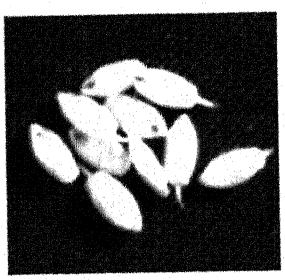
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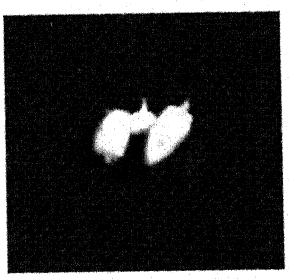
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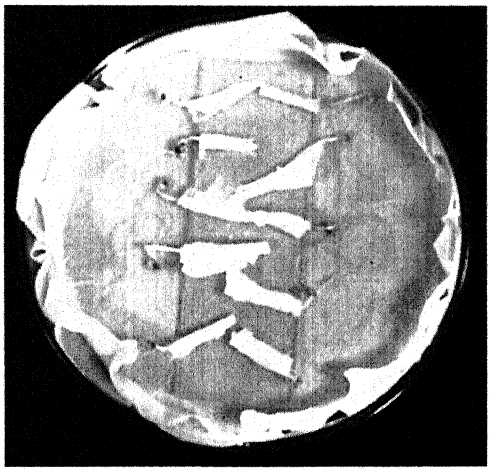
PLATE 5: INDUCTION OF TETRAPLOIDY IN DIPLOID MALE STERILE PEARL MILLET LINES

1. Colchicine treatment to shoot (shoot treatment)
2. 81A1 (2x) seeds
3. 81A1 (induced tetraploid) seeds
4. T1 seeds
5. 81A4 (2x) seeds
6. 81A4 (induced tetraploid) seeds
7. T1 seeds
8. Spike showing numerous seeds in C_1 generation
9. Induced tetraploid of 81A1 (C_0 generation)
10. Diploid 81A1 (initiation of flowering)
11. Induced tetraploid of 81A1 (C_1 generation, no initiation of flowering)
12. T1
13. Induced tetraploid of 81A4 (C_0 generation)
14. Diploid 81A4 (flowering)
15. Induced tetraploid of 81A4 (C_1 generation, no initiation of flowering)
16. Stomata 81A1 (2x)
17. Stomata 81A1 (induced tetraploid)
18. Stomata 81A4 (2x)
19. Stomata 81A4 (induced tetraploid)

PLATE 5: INDUCTION OF TETRAPLOIDY IN DIPLOID MALE STERILE PEARL MILLET LINES



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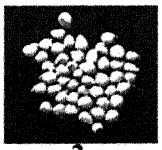

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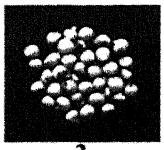

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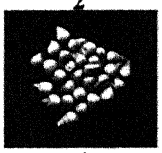

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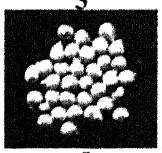

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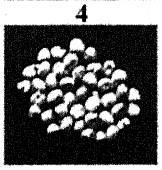

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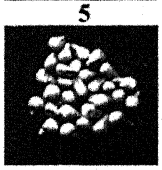

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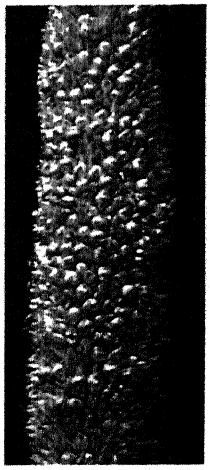

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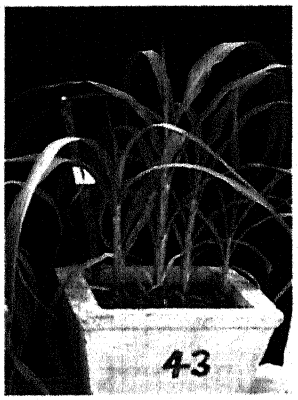

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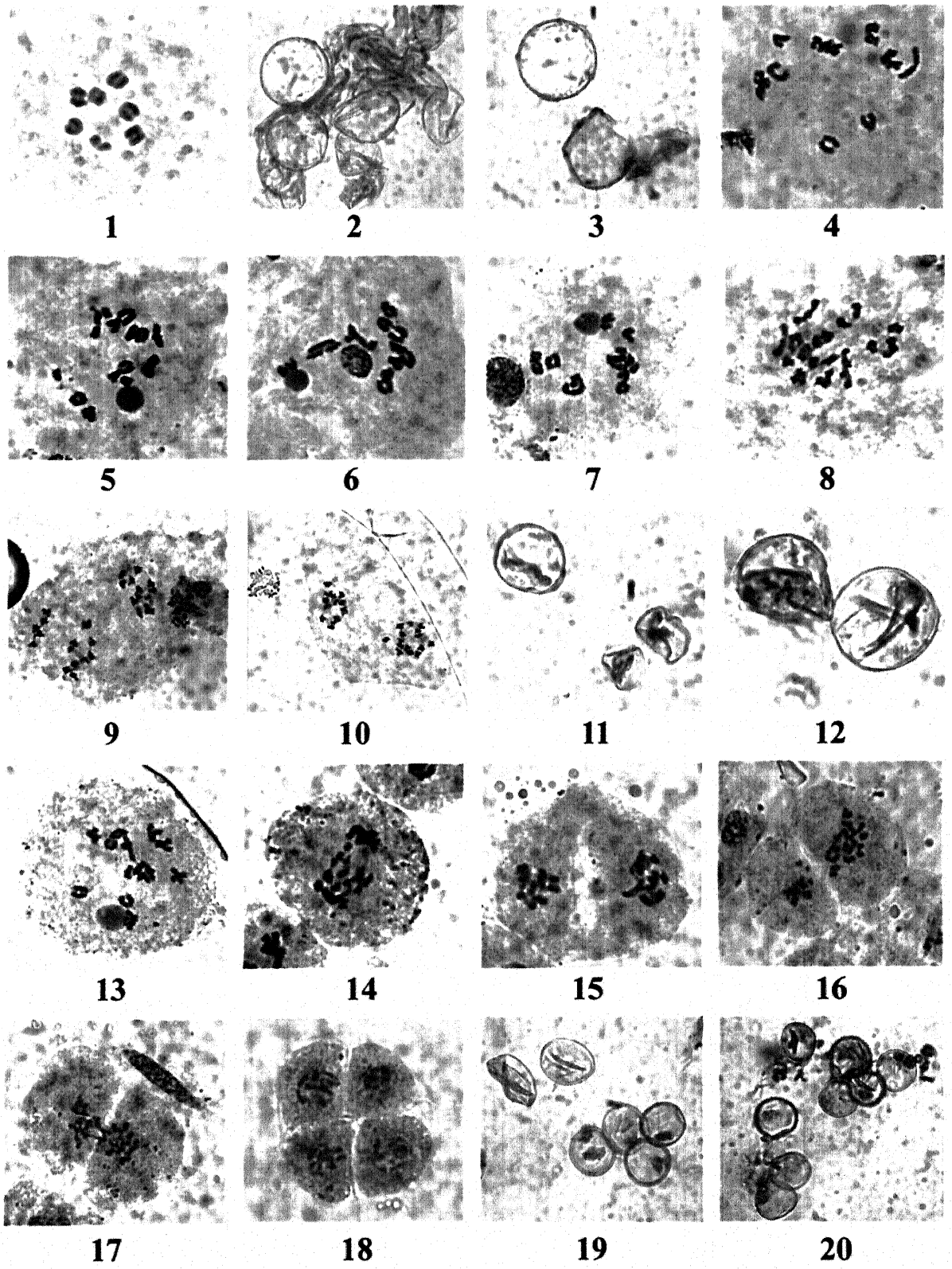
PLATE 6: CYTOLOGICAL OBSERVATIONS IN DIPLOID AND INDUCED TETRAPLOID MALE STERILE PEARL MILLET

1. Diploid pearl millet (81A1 male sterile) diakinesis 7_{II}.
2-3. Diploid pearl millet (81A1 male sterile) pollen.
4. Induced tetraploid of 81A1 (diakinesis 14_{II}).
5. Induced tetraploid of 81A1 (diakinesis $2_I + 13_{II}$).
6. Induced tetraploid of 81A1 (diakinesis $16_{II} + 4_{IV}$).
7. Induced tetraploid of 81A1 (diakinesis $10_{II} + 2_{IV}$).
8. Induced tetraploid of 81A1 (metaphase I).
9. Induced tetraploid of 81A1 (telophase I 14:14).
10. Induced tetraploid of 81A1 (telophase I 12:16).
11-12. Induced tetraploid of 81A1 non viable pollen.
13. Induced tetraploid of 81A4 (diakinesis $2_I + 13_{II}$).
14. Induced tetraploid of 81A4 (anaphase I).
15-16. Induced tetraploid of 81A4 (dyad).
17. Induced tetraploid of 81A4 (dyad with chromosome bridges).
18. Induced tetraploid of 81A4 (tetrad).
19-20. Induced tetraploid of 81A4 non viable pollen.

PLATE 6: CYTOLOGICAL OBSERVATIONS IN DIPLOID AND INDUCED TETRAPLOID MALE STERILE PEARL MILLET



1 2 3 4

5 6 7 8

9 10 11 12

13 14 15 16

17 18 19 20

PLATE 7: EMBRYO RESCUE IN INTERSPECIFIC CROSSES OF *PENNISETUM*

1. Control (T1) and Hybrid (T1 x *P. pedicellatum* Agros 4 (germination difference).
2. Two weeks old control plantlets.
3. Four weeks old plantlets.
4. Hybrids (pearl millet x *P. polystachyon* and pearl millet x *P. pedicellatum*).
5. Hybrids (pearl millet x *P. pedicellatum*).
6. Hybrids (pearl millet x *P. pedicellatum*) and control plantlets (from right to left).
7. Hybrid pearl millet x *P. pedicellatum* (10 weeks).
8. Hybrids (pearl millet x *P. polystachyon* (10 weeks).
9. *In vitro* flowering in T1 (10 weeks).

PLATE 7: EMBRYO RESCUE IN INTERSPECIFIC CROSSES OF *PENNISETUM*

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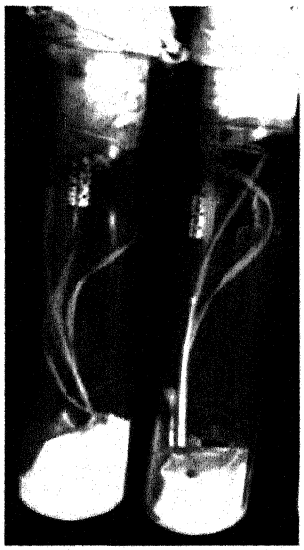

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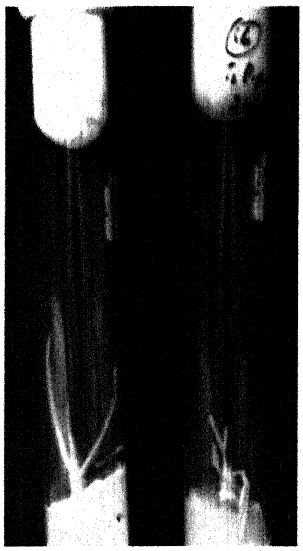

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PLATE 8: MORPHOLOGY OF PEARL MILLET (4x) X *P. SQUAMULATUM* F_1 AND BC_1 HYBRIDS

1. Pearl millet (T1).
2. Spikes (from left to right: T1, *P. squamulatum* IG 98-360, H1, H2, BC_1 (last five spikes).
3. Spikes (from left to right: T1, *P. squamulatum* IG 98-360, H1, H2, BC_1 (last two spikes).
4. Node color and hairiness variation in parents, F_1 and BC_1 (from left to right: first four spikes of BC_1, H1, H2, *P. squamulatum* IG 98-360, T1).
5. Violet coloured stigma of *P. squamulatum* and white coloured stigma of pearl millet (T1).
6. Variation in stigma colour (from left to right: violet stigma of *P. squamulatum*, white of T1, white of H1 and violet of H2).
7. Trifid and violet coloured stigma of *P. squamulatum.*
8. Stigma colour variation in BC_1 plants.
9. Morphology pearl millet (T2).
10. *P. squamulatum* IG 98-360.
11. *P. squamulatum* IG 2000-36.
12. H1.
13. H2.
14. H3.
15. H4.
16. H5.
17-23. Variation in morphology of BC_1 plants.

PLATE 8: MORPHOLOGY OF PEARL MILLET (4x)X *P. SQUAMULATUM* F_1 AND BC_1 HYBRIDS

PLATE 9: CYTOLOGICAL OBSERVATIONS IN PEARL MILLET, *P. SQUAMULATUM* AND THEIR HYBRIDS

1. *P. glaucum* (T1) diakinesis ($8_{II} + 3_{IV}$)
2. *P. glaucum* (T1) metaphase I
3. *P. glaucum* (T1) pollen
4. *P. squamulatum* (IG 98-360) diakinesis ($15_{II} + 2_{III} + 5_{IV}$)
5. *P. squamulatum* (IG 98-360) diakinesis ($20_{II} + 4_{IV}$)
6. *P. squamulatum* (IG 98-360) metaphase ($14_{II} + 7_{IV}$)
7. *P. squamulatum* (IG 98-360) metaphase ($1_I + 13II + 6_{IV} + 1_V$)
8. *P. squamulatum* (IG 98-360) metaphase ($15_{II} + 1_{III} + 3_{IV} + 1_V + 1_{IV}$)
9. *P. squamulatum* (IG 98-360) anaphase I (2n=56)
10. *P. squamulatum* (IG 98-360) mitosis (metaphase 2n=56)
11. *P. squamulatum* (IG 2000-36) diakinesis ($26_{II} + 1_{IV}$)
12. *P. squamulatum* (IG 2000-36) pollen
13. F_1 (H1) diakinesis (21_{II})
14. F_1 (H1) diakinesis ($2_I + 20_{II}$)
15. F_1 (H1) dyad (21 + 21)
16. F_1 (H1) pollen
17. F_1 (H2) diakinesis ($17_{II} + 2_{IV}$)
18. F_1 (H2) diakinesis ($2_I + 18_{II} + 1_{IV}$)
19. F_1 (H2) diakinesis (21_{II})
20. F_1 (H2) dyad (22 + 20)

PLATE 9: CYTOLOGICAL OBSERVATIONS IN PEARL MILLET, *P. SQUAMULATUM* AND THEIR HYBRIDS

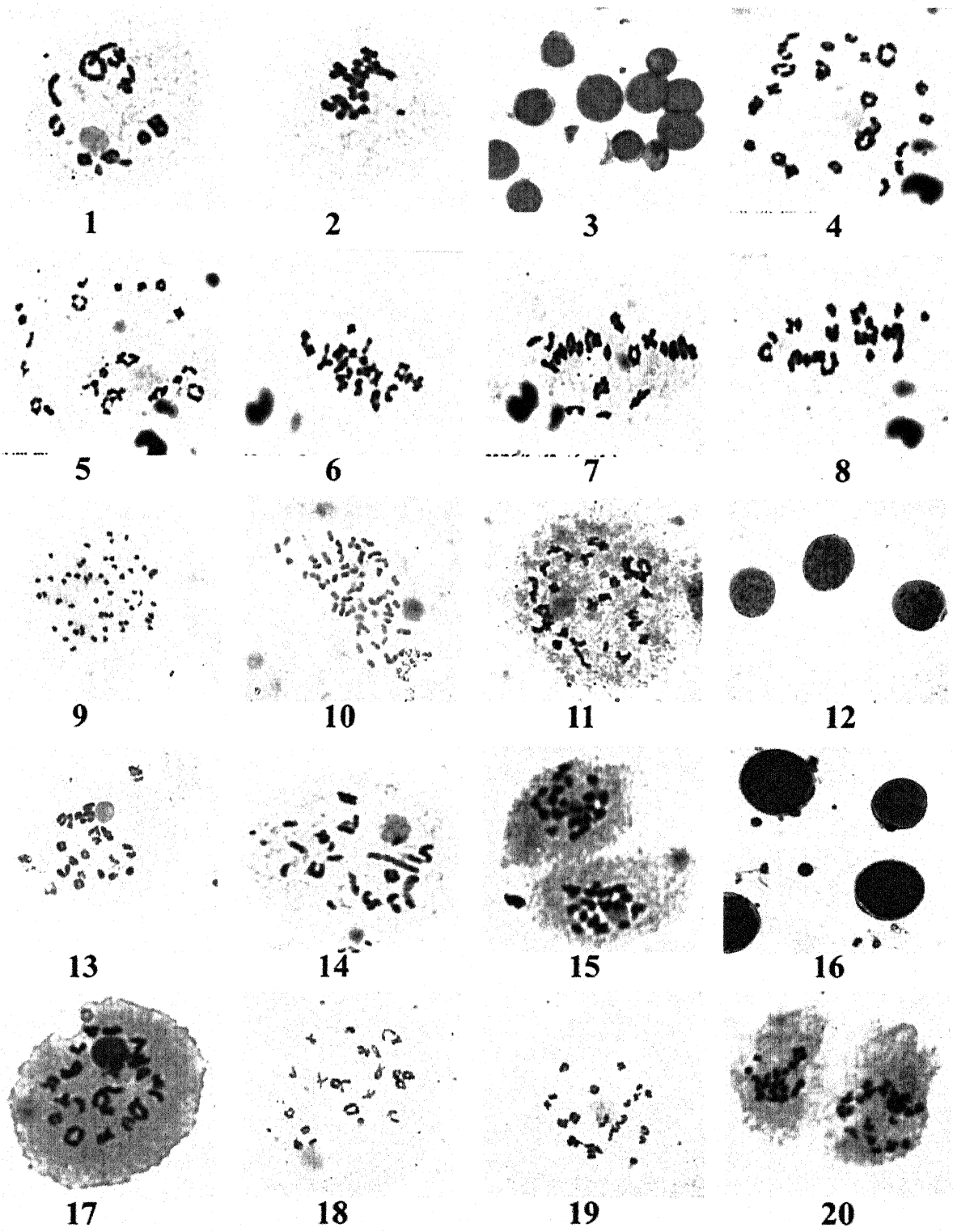
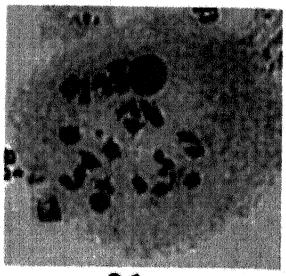


PLATE 9: (Contd.)

21. H1 diakinesis.
22. H1 dyad (21:21).
23. H1 pollen (showing high fertility and size difference) 83.9 %.
24. Hybrid 2 (H2) diakinesis 21_{II}.
25. H2 diakinesis (4I + 15II + 2IV).
26. H2 diakinesis (21_{II}).
27. H2 dyad.
28. H2 tetrad (equal distribution of chromosomes).
29. H2 pollen (63.7 % fertility).
30. H3 diakinesis ($2_I + 20_{II}$).
31. H3 diakinesis ($4_I + 19_{II}$).
32. H3 metaphase I.
33. H3 pollen (29.2% fertitlity).
34. H4 diakinesis ($2_I + 18_{II} + 1_{IV}$).
35. H4 diakinesis (21_{II}).
36. H4 anaphase I (21:21).
37. H4 pollen (30.8% fertility).
38. H5 diakinesis 21_{II}.
39. H5 dyad (21:21).
40. H5 pollen (73.6 % fertility).

PLATE 9: (Contd.)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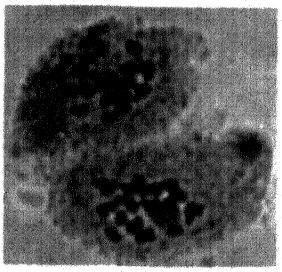
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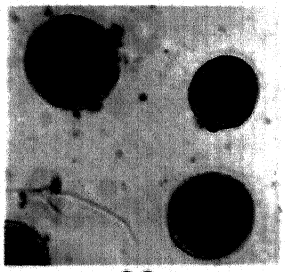
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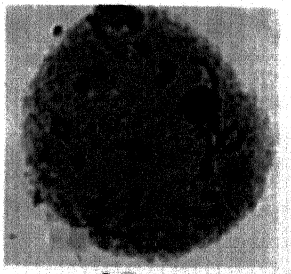
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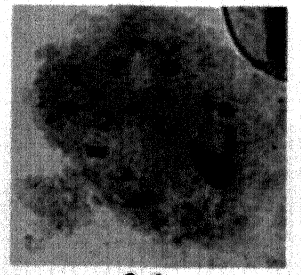
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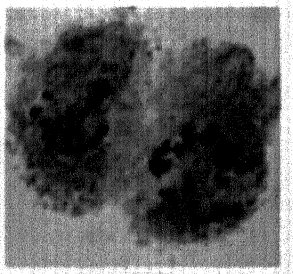
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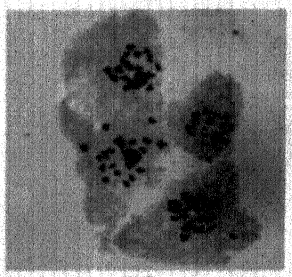
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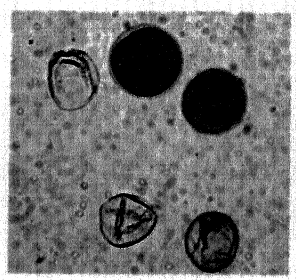
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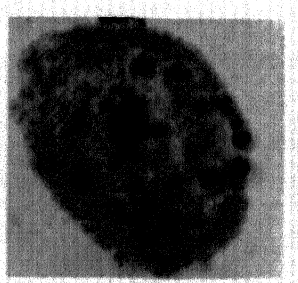
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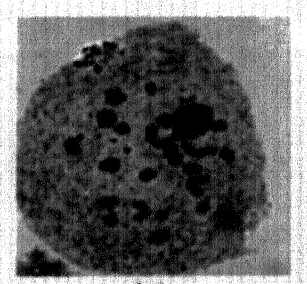
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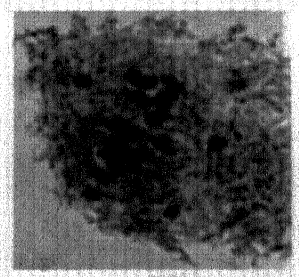
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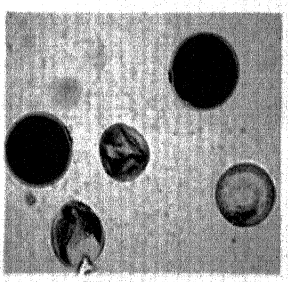
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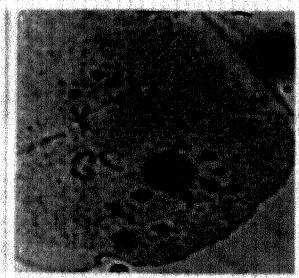
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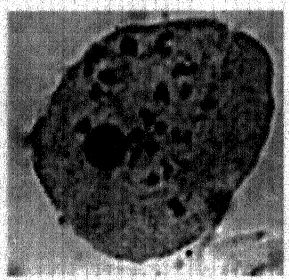
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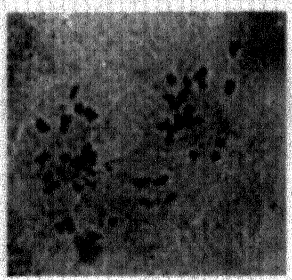
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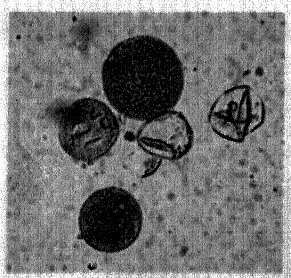
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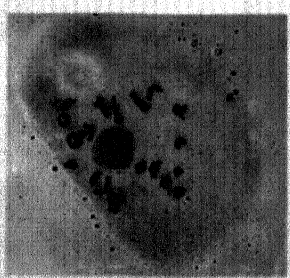
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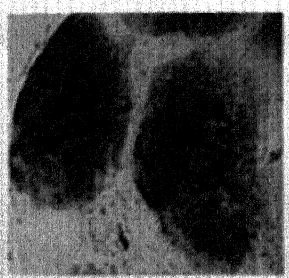
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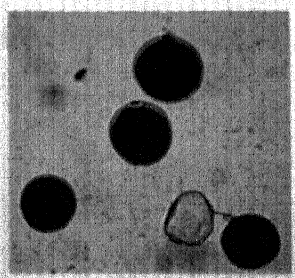
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PLATE 10: CYTOLOGICAL OBSERVATIONS IN BC_1 PLANTS (PEARL MILLET (4x) X *P. SQUAMULATUM*)

1. H1T1 diakinesis ($15_{II} + 1_{IV}$).
2. H1T1 anaphase I (2n=35).
3. H1T1 Tripolar anahase I (non-orientation of chromosomes).
4. H1T1 metaphase I ($8_I + 9_{II} + 3_{III}$).
5. H1T1 Tripolar anahase I.
6. H1T1Tripolar anahase I with three laggards.
7. H1T1 non orientation of chromosomes at telophase I.
8. H1T1 hexad with two microcytes.
9. H1T1 hexad with five equal sized microspores.
10. H1T1 hexad with two microcytes.
11. H1T1 pollen (sterile).
12. T1H1 diakinesis ($2_I + 15_{II} + 1_{III}$).
13. T1H1 anaphase I (2n=35).
14. T1H1 non viable pollen showing complete sterility.
15. H1T2 diakinesis ($3_I + 16_{II}$).
16-17. H1T2 tetrad with microcytes.
18. H1T2 pollen non viable showing complete sterility.
19. T2H1 diakinesis ($5_I + 15_{II}$).
20. T2H1 diakinesis with 8 univalents.

PLATE 10: CYTOLOGICAL OBSERVATIONS IN BC_1 PLANTS (PEARL MILLET (4x) X *P. SQUAMULATUM*)

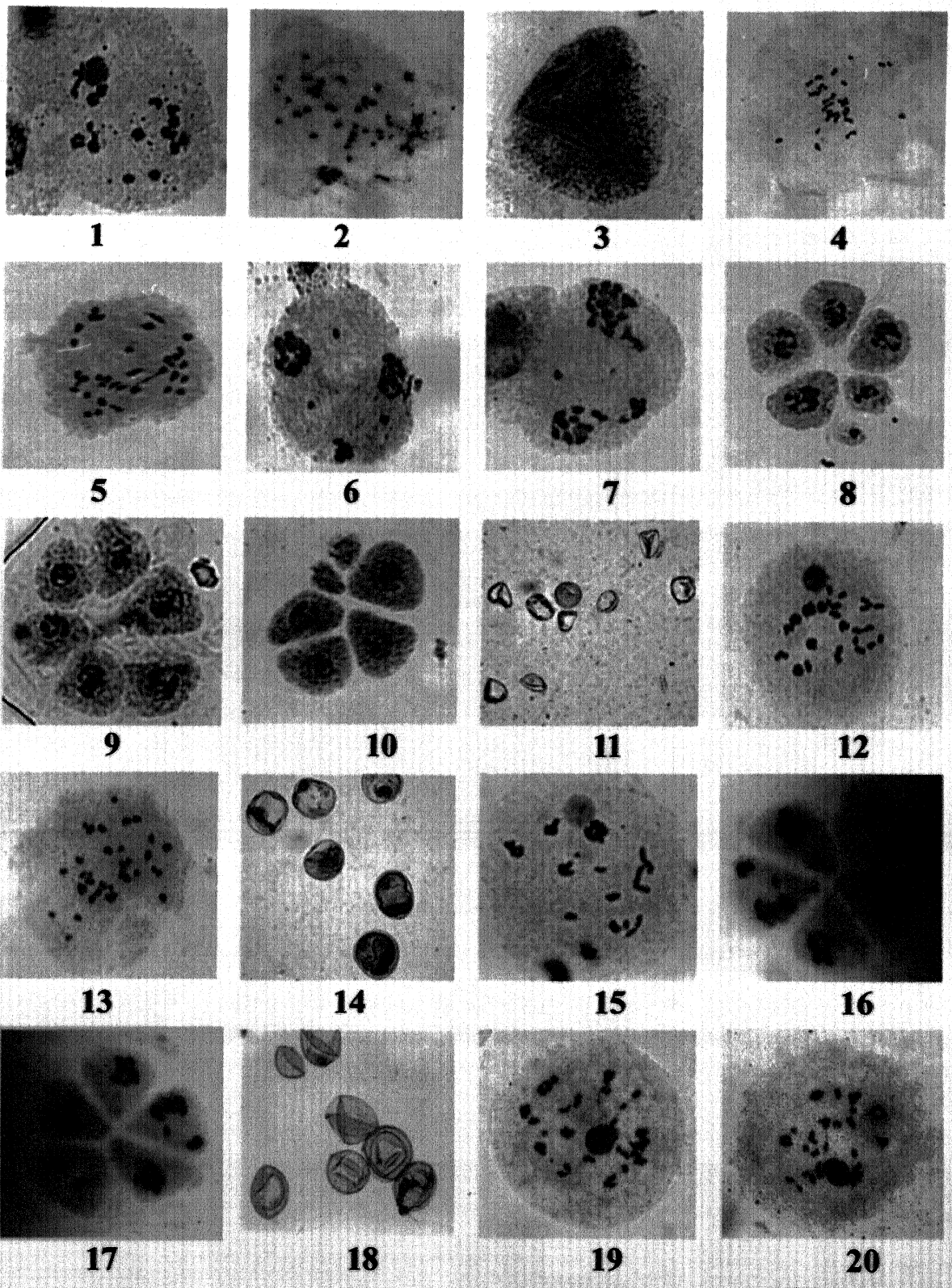
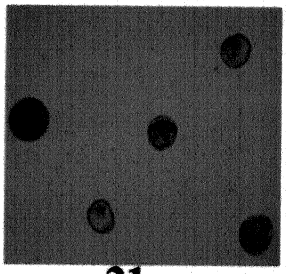


PLATE 10: (Contd.)

21-22. T2H1 (Pollen-variation in size and fertility of different plants of this cross).
23. T1H1 diakinesis.
24. T1H2 metaphase I ($10_I + 11_{II} + 1_{III}$).
25. T1H2 metaphase I ($8_I + 12_{II} + 1_{III}$) 8 univalents were counted in the metaphase I.
26. T1H2 anaphase I 2n=35 (20:15).
27. T1H2 dyad (22:13).
28. T1H2 metaphase II showing precocious division in one microcyte.
29. T1H2 pollen (14 % fertility).
30. T1H2 pollen (sterile).
31. T2H2 diakinesis ($7_I + 12_{II} + 1_{IV}$).
32. T2H2 diakinesis ($6_I + 13_{II} + 1_{III}$).
33-34. T2H2 diakinesis ($5_I + 15_{II}$).
35. T2H2 diakinesis ($8_I + 11_{II} + 1_{IV}$).
36. T2H2 tetrad showing chromosome bridges.
37. T2H2 meiotic product with micro nuclei in microspore.
38-40. Variation in pollen fertility in different plants of same cross (T2H2).

PLATE 10: (Contd.)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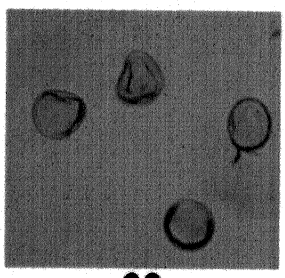
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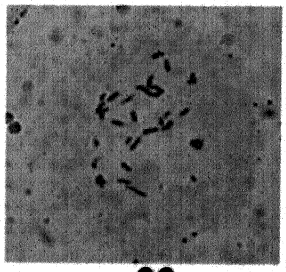
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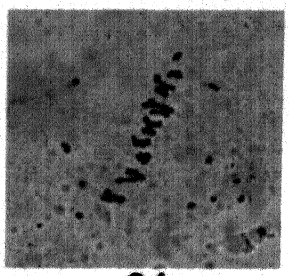
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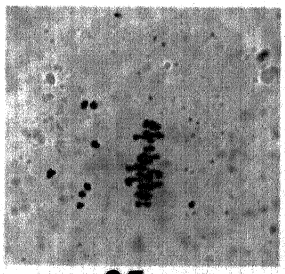
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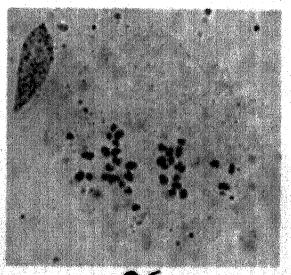
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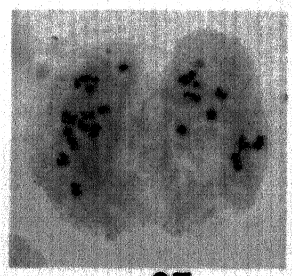
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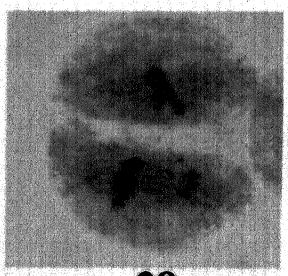
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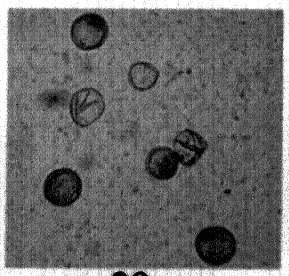
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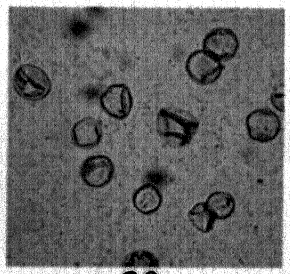
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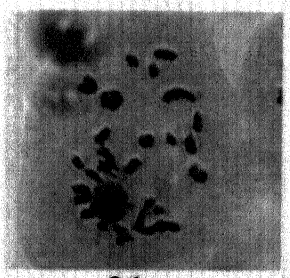
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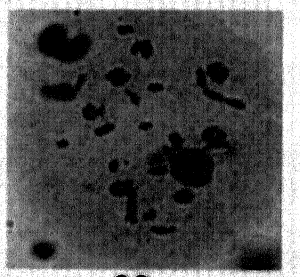
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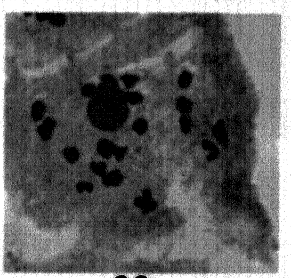
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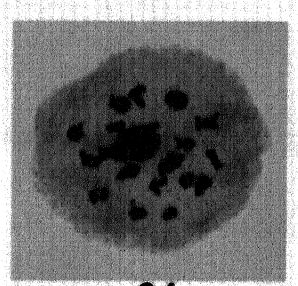
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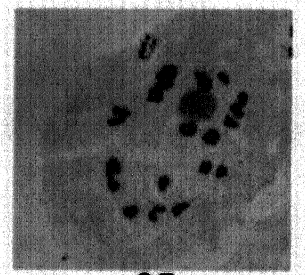
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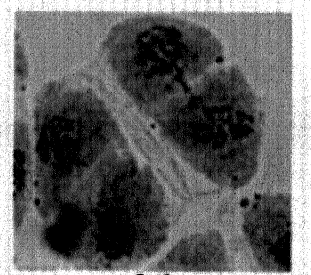
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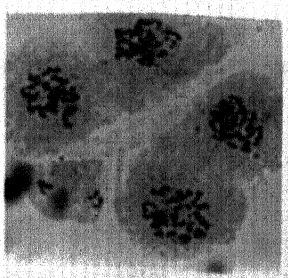
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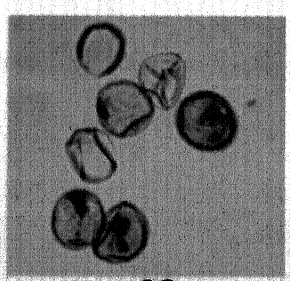
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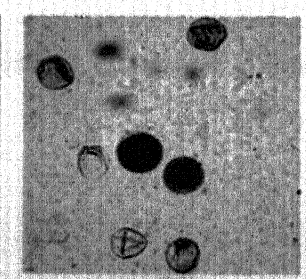
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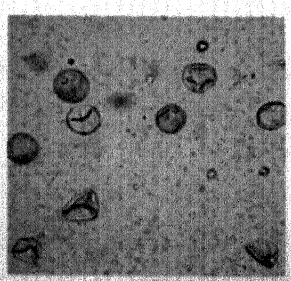
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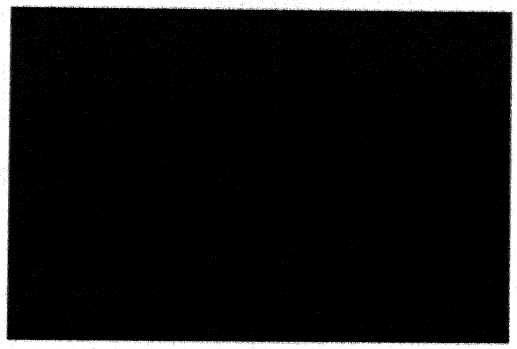
PLATE 11: ISOZYMES AND NATIVE PROTEIN IN PEARL MILLET AND P. SQUAMULATUM F_1 AND BC_1 HYBRIDS

1. Esterase (samples from left to right)
   P. squamulatum (IG 98-360), H1, *P. glaucum* (T2), BC_1 (H1T2 MP 5/26, MP 2/18, MP 2/32, MP 4/30, MP 4/7, MP 4/3, MP 2/17).
2. Esterase (samples from left to right)
   P. squamulatum (IG 98-360), H2, *P. glaucum* (T1), T2, BC_1 (T2H2 MP 4/15, MP 4/14, MP 4/12, MP 2/24), (T1H2 MP 4/10, MP 2/20, MP 2/21), (T1H1 MP 5/35), H1.
3. Superoxide desmutase (samples from left to right)
   P. squamulatum (IG 98-360), H1, *P. glaucum* (T2), BC_1 (H1T2 MP 5/26), (T2H1 MP 2/18, MP 2/32, MP 4/30, MP 4/7, MP 4/3, MP 2/17, MP 4/4, MP 4/31, MP 4/6).
4. Superoxide desmutase (samples from left to right)
   P. squamulatum (IG 98-360), H2, *P. glaucum* (T1), T2, BC_1 (T2H2 MP 4/15, MP 4/14, MP 4/12, MP 2/24), (T1H2 MP 4/10, MP 2/20, MP 2/21), (T1H1 MP 5/35), H1.
5. Peroxidase (samples from left to right)
   P. squamulatum (IG 98-360), H1, *P. glaucum* (T2), BC_1 (H1T2 MP 5/26, MP 2/18, MP 2/32, MP 4/30, MP 4/7, MP 4/3, MP 2/17).
6. Peroxidase (samples from left to right)
   P. squamulatum (IG 98-360), H2, *P. glaucum* (T1), T2, BC_1 (T2H2 MP 4/15, MP 4/14, MP 4/12, MP 2/24), (T1H2 MP 4/10, MP 2/20, MP 2/21), (T1H1 MP 5/35), H1.
7. Native protein (samples from left to right)
   P. squamulatum (IG 98-360), H1, *P. glaucum* (T2), BC_1 (H1T2 MP 5/26, MP 2/18, MP 2/32, MP 4/30, MP 4/7, MP 4/3, MP 2/17).
8. Native protein (samples from left to right)
   P. squamulatum (IG 98-360), H2, *P. glaucum* (T1), T2, BC_1 (T2H2 MP 4/15, MP 4/14, MP 4/12, MP 2/24), (T1H2 MP 4/10, MP 2/20, MP 2/21), (T1H1 MP 5/35), H1.

PLATE 11: ISOZYMES AND NATIVE PROTEIN IN PEARL MILLET AND *P. SQUAMULATUM* F_1 AND BC_1 HYBRIDS



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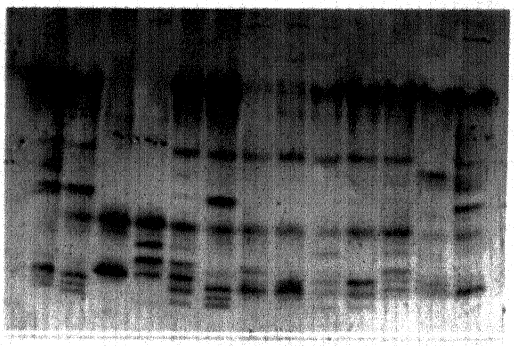

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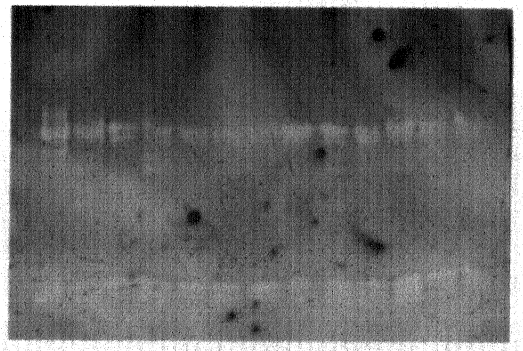

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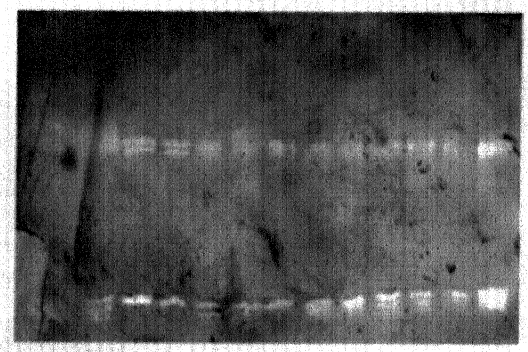

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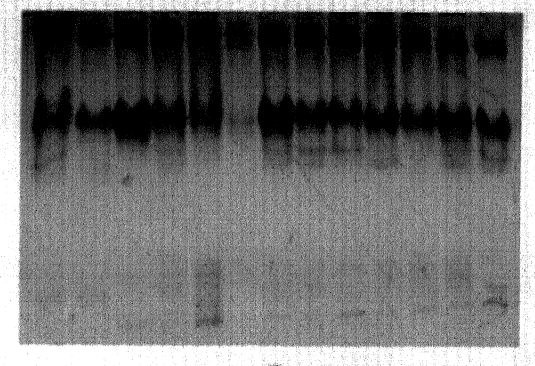

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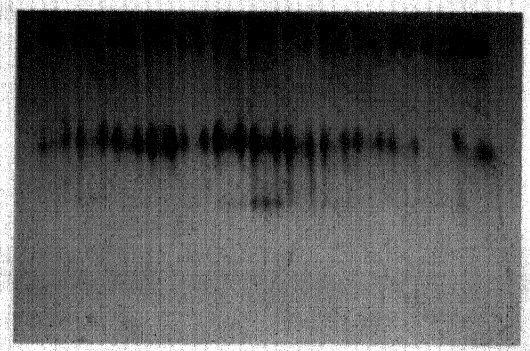

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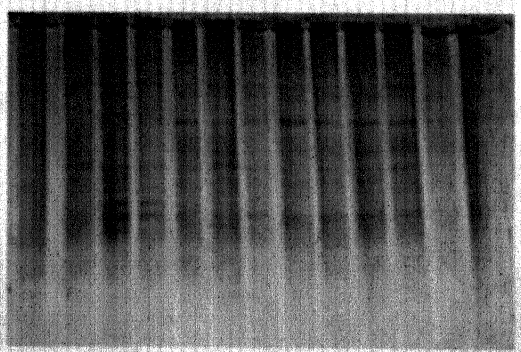

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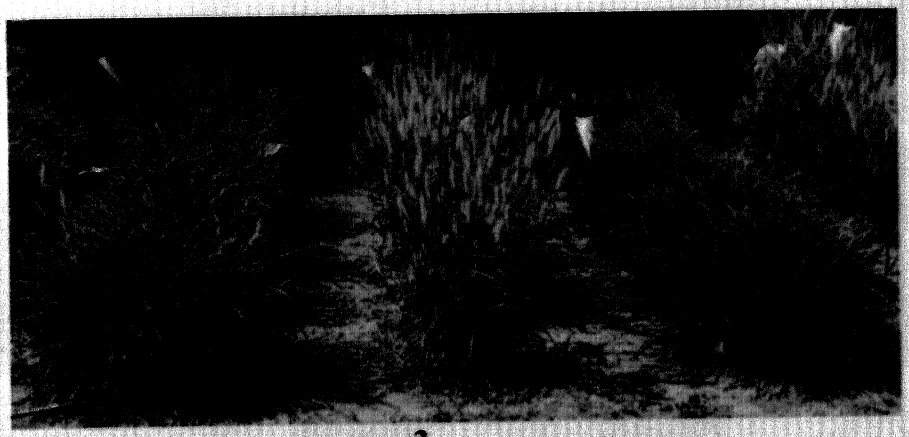
PLATE 12: MORPHOLOGY OF GO AND GOS HYBRIDS

1 and 3. Lines 1 and 3 are F_1 hybrids showing late flowering then BC_1 in lines 2 and 4.
2. F_1 hybrid.
4. Hybrid 1 (BC_1 x T1) turned out to be an annual.
5. Hybrid 2 (BC_1 x T2) turned out to be an annual.
6. Hybrid 3 (BC_1 x T2) perennial.
7. GOS Hybrid 4 (BC_1 x H1) perennial.
8. GOS Hybrid 5 & 6 (BC_1 x H2) perennial.
9. GOS Hybrid 7 (BC_1 X H2) perennial.
10. GOS Hybrid 8 (BC_1 X H2) perennial.
11. GOS Hybrid 9(BC_1 X H2) perennial.
12. Hybrid 10 (diploid pearl millet 81 A4 x H2) annual.

PLATE 12: MORPHOLOGY OF GO AND GOS HYBRIDS

1

2



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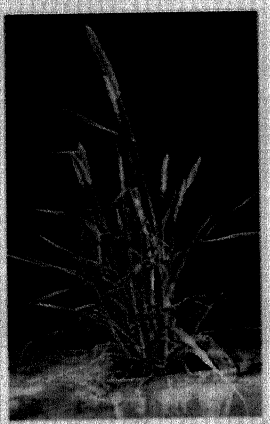

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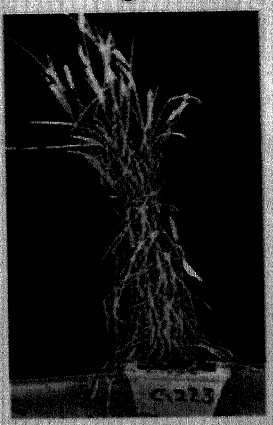

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PLATE 13: MEIOSIS IN PEARL MILLET, *P. ORIENTALE* AND THEIR F_1 AND BC_1 HYBRIDS

1. Pearl millet (81B) diakinesis (7_{II}).
2. Pearl millet pollen.
3. *P. orientale* (IG 04-165) diakinesis (2n=54, $23_{II} + 2_{IV}$).
4. *P. orientale* pollen.
5-6. F_1 (Hybrid) diakinesis (2n=16, 7G +9O).
7. F_1 binucleolate pollen mother cell.
8. F_1 diakinesis (2n=32, precocious division of chromosomes).
9. F_1 diakinesis (3 micronucleoli).
10. F_1 microsporocyte (showing few condensed chromosomes).
11. F_1 microsporocyte (showing cytomixis at early prophase).
12. F_1 pollen mitosis.
13. F_1 pollen mitosis (2n=16).
14. BC_1 diakinesis $7_{II}G + (5_I + 2_{II})O$.
15-16. BC_1 metaphase I (9 univalents of *P. orientale* can be invariable seen).
17. BC_1 telophase I.
18. BC_1 prophase II (multiple micronuclei).
19. BC_1 tetrad.
20. BC_1 tetrad with pycnotic micronuclei.

(When bridges broke in anaphase and telophase I, they gave rise to micronuclei that persisted in pycnotic form in later stages).

PLATE 13: MEIOSIS IN PEARL MILLET, *P. ORIENTALE* AND THEIR F_1 AND BC_1 HYBRIDS

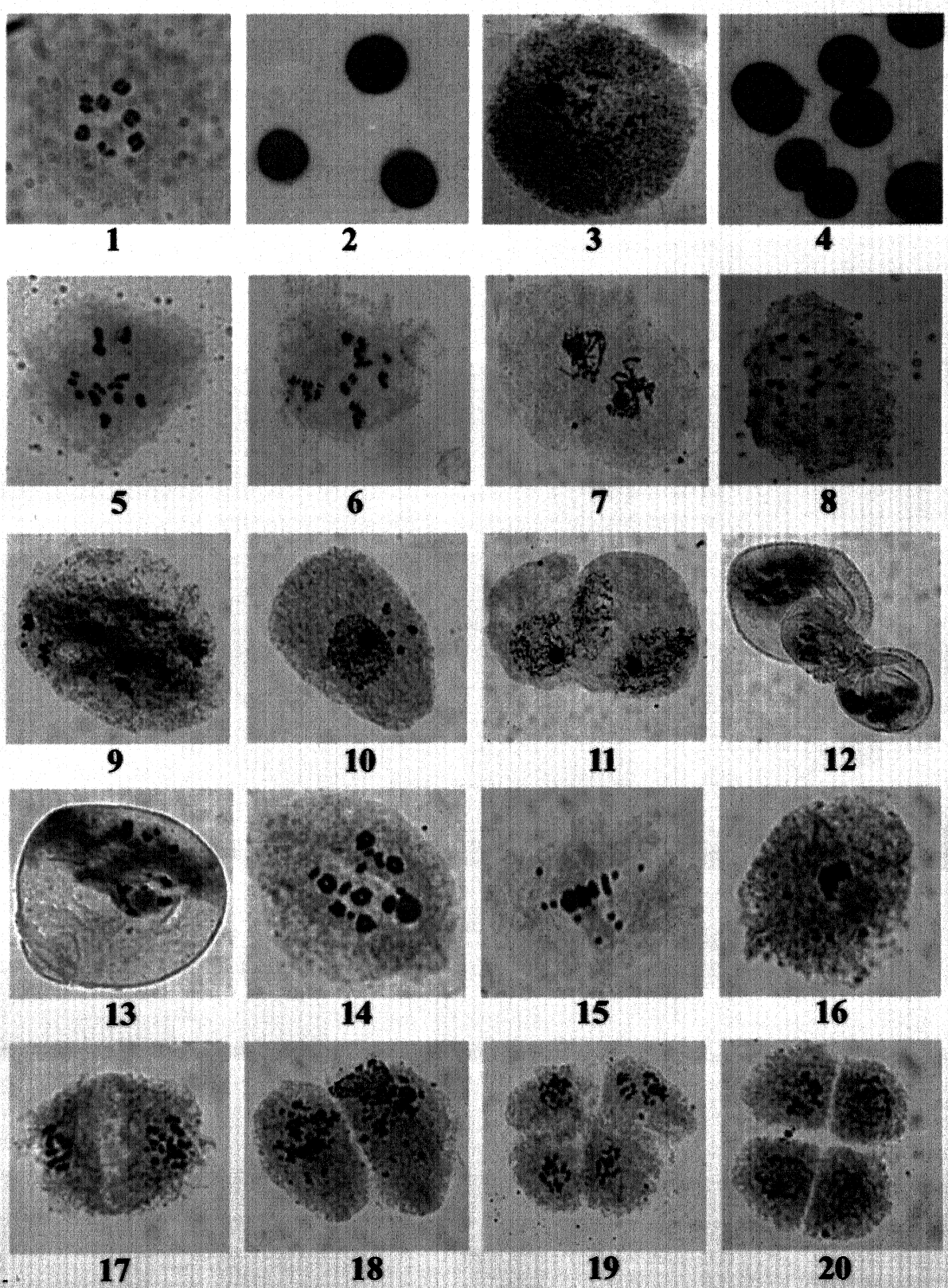


PLATE 14: MEIOSIS IN PARENTS AND BC_1 (GO) PLANT 2

1. F_1 (GO) diakinesis 2n=16, $7_{I}G + (7_{I} + 1_{II})O$.
2. Pearl millet (diploid) diakinesis 2n=14.
3. BC_1 (GO) a uninucleolate microsporocyte at pachytene.
4- 6. BC_1 (GO) a binucleolate microsporocyte at pachytene.
7. BC_1 (GO) diakinesis 2n=23 $(8_{I} + 3_{II})G + (7_{I} + 1_{II})O$.

Aspects of meiocytes that undervent microsporogenesis in mature anthers:

8-9. Prophase meiocyte found among normal pollen grains before entering meiosis. Note the well-developed exine wall.

10-20. Diakinesis.

10. $7_{II}G + 9_{I}O$.
11. $7_{II}G + (3_{I} + 3_{II})O$.
12. $7_{II}G + (4_{II} + 1_{I})O$.
13. $7_{II}G + (3_{I} + 3_{II})O$.
14. $7_{II}G + (3_{I} + 1_{II})O$.
15. $7_{II}G + (3_{I} + 5_{II})O$.
16. $7_{II}G + (2_{I} + 4_{II})O$.
17. $7_{II}G + (3_{I} + 3_{II})O$.
18. $7_{II}G + 7_{I}O$.
19. $(2_{I} + 7_{II})G + (4_{I} + 1_{II})O$.
20. $7_{II}G + (7_{I} + 1_{II})O$.

PLATE 14: MEIOSIS IN PARENTS AND BC_1 (GO) PLANT 2

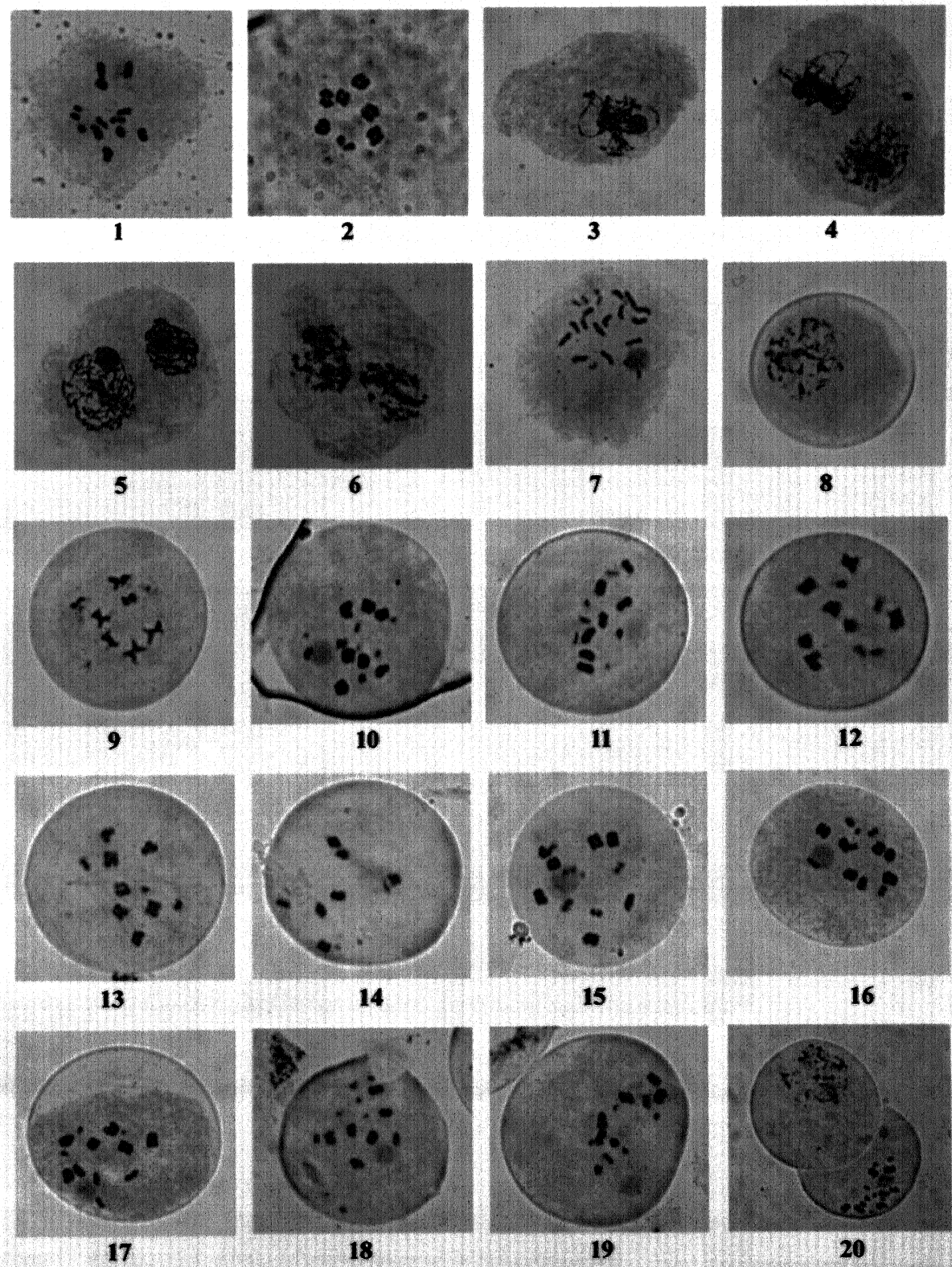


PLATE 15: CYTOLOGICAL OBSERVATIONS IN BC_1 (GO) PLANT 2

1-4. Two to three cells linked together exhibiting cytomixis.
5. One cell showing diakinesis [(2I + 7II)G + (3I + 4II)O] and one cell at leptotene.
6. Two cells linked together with one empty cell.
7. Cells attached together showing different chromosome numbers.
8-16. Note the cytoplasmic channel between two cells.
17-20. Cells showing anaphase and telophase.

PLATE 15: CYTOLOGICAL OBSERVATIONS IN BC_1 (GO) PLANT 2

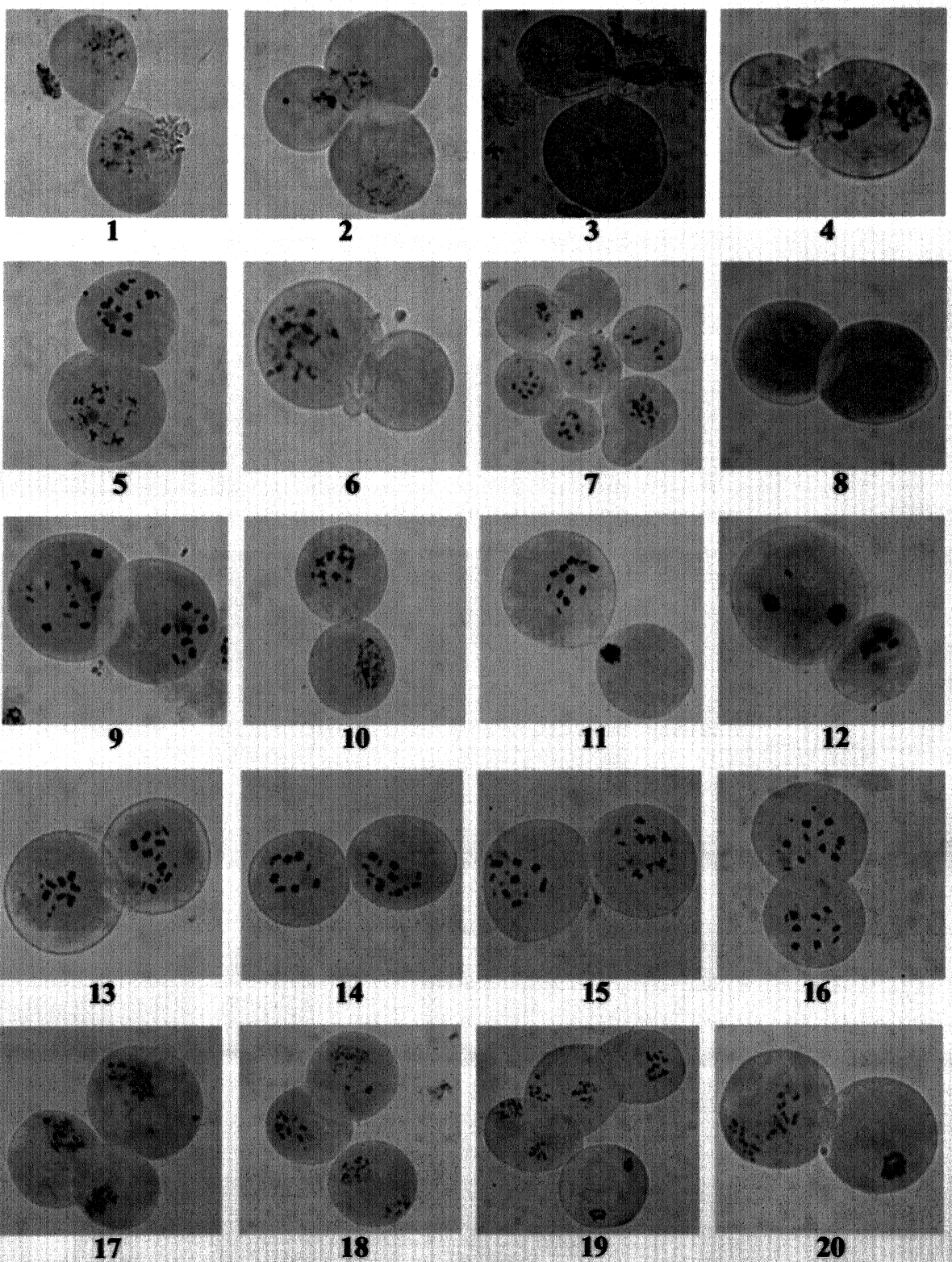


PLATE 16: MEIOSIS IN [BC1 (GO) X PEARL MILLET (4x)] HYBRIDS

1. BC_1 (GO): diakinesis ($7_{II}G + 9_IO$).
2. BC_1 (GO): metaphase I with 9 smaller univalents (of *P. orientale*).
3. Pearl millet (T1): diakinesis ($4_I + 4_{II} + 4_{IV}$).
4. Pearl millet (T1): metaphase I ($2_{II} + 5_{IV}$).
5. Pearl millet (T1): pollen.
6. Hybrid 1: diakinesis ($4_I + 10_{II} + 3_{III} + 1_{IV}$).
7. Hybrid 1: diakinesis ($4_I + 13_{II} + 1_{III} + 1_{IV}$).
8. Hybrid 1: diakinesis ($3_I + 13_{II} + 2_{IV}$).
9. Hybrid 1: metaphase I (9 univalents of *P. orientale* are invariably seen).
10. Hybrid 1: anaphase I.
11. Hybrid 1: pollen (sterile).

12-13. Hybrid 2: diakinesis ($4_I + 13_{II} + 1_{III} + 1_{IV}$).

14. Hybrid 2: metaphase I (7 univalents of *P. orientale*).
15. Hybrid 2: pollen (sterile).
16. Hybrid 3: diakinesis ($1_I + 12_{II} + 3_{IV}$).
17. Hybrid 3: diakinesis 5_{IV} (note the interlocking present).
18. Hybrid 3: diakinesis ($1_I + 15_{II} + 2_{III}$).
19. Hybrid 3: metaphase I.
20. Hybrid 3: pollen (sterile).

PLATE 16: MEIOSIS IN [BC_1 (GO) X PEARL MILLET(4x)] HYBRIDS

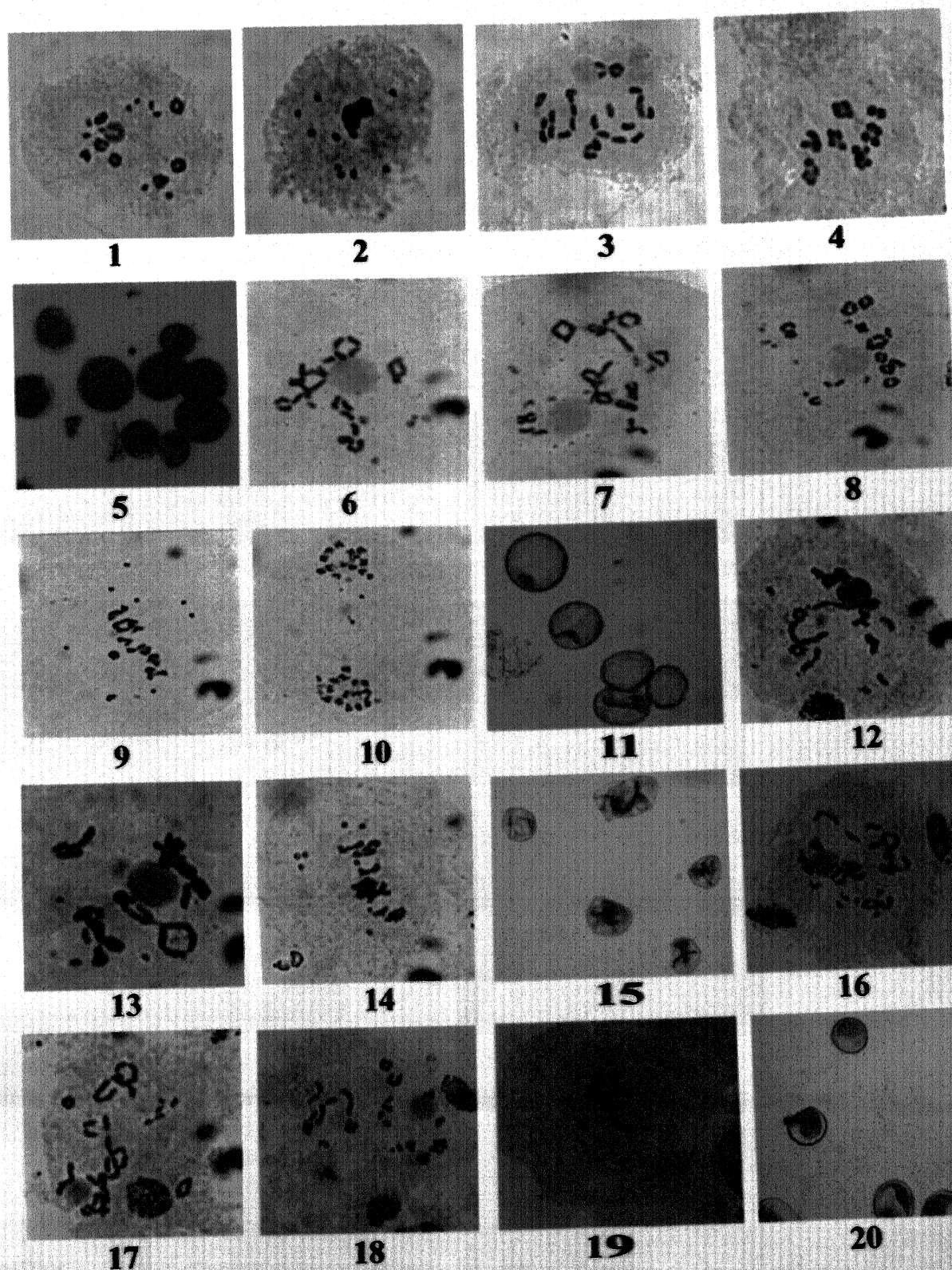


PLATE 17: MEIOSIS IN GOS HYBRID 4

1. BC_1 (GO) diakinesis $7_{II}G + 9_IO$.
2. BC_1 (GO) diakinesis $7_{II}G + (5_I + 2_{II})O$.
3. H1 (pearl millet x *P. squamulatum*) diakinesis 21_{II}.
4. H1 (pearl millet x *P. squamulatum*) pollen.
5. GOS Hybrid 4 diakinesis ($10_I + 14_{II} + 2_{III}$).
6. GOS Hybrid 4 diakinesis ($12_I + 11_{II} + 2_{III} + 1_{IV}$).
7-8. GOS Hybrid 4 diakinesis (all univalents).
9. GOS Hybrid 4 dyad.
10. GOS Hybrid 4 anaphase II.
11. GOS Hybrid 4 (non orientation of chromosomes at anaphase II).
12-13. GOS Hybrid 4 (four nucleate and three nucleate coenocytic microspore).
14-15. GOS Hybrid 4 (non orientation of chromosomes at anaphase I).
16-17. GOS Hybrid 4 diakinesis (note the well-developed exine wall while diakinesis still going on similar to the female parent).
18-19. GOS Hybrid 4 (tetrad and pentad).
20. GOS Hybrid 4 pollen (sterile).

PLATE 17: MEIOSIS IN GOS HYBRID 4

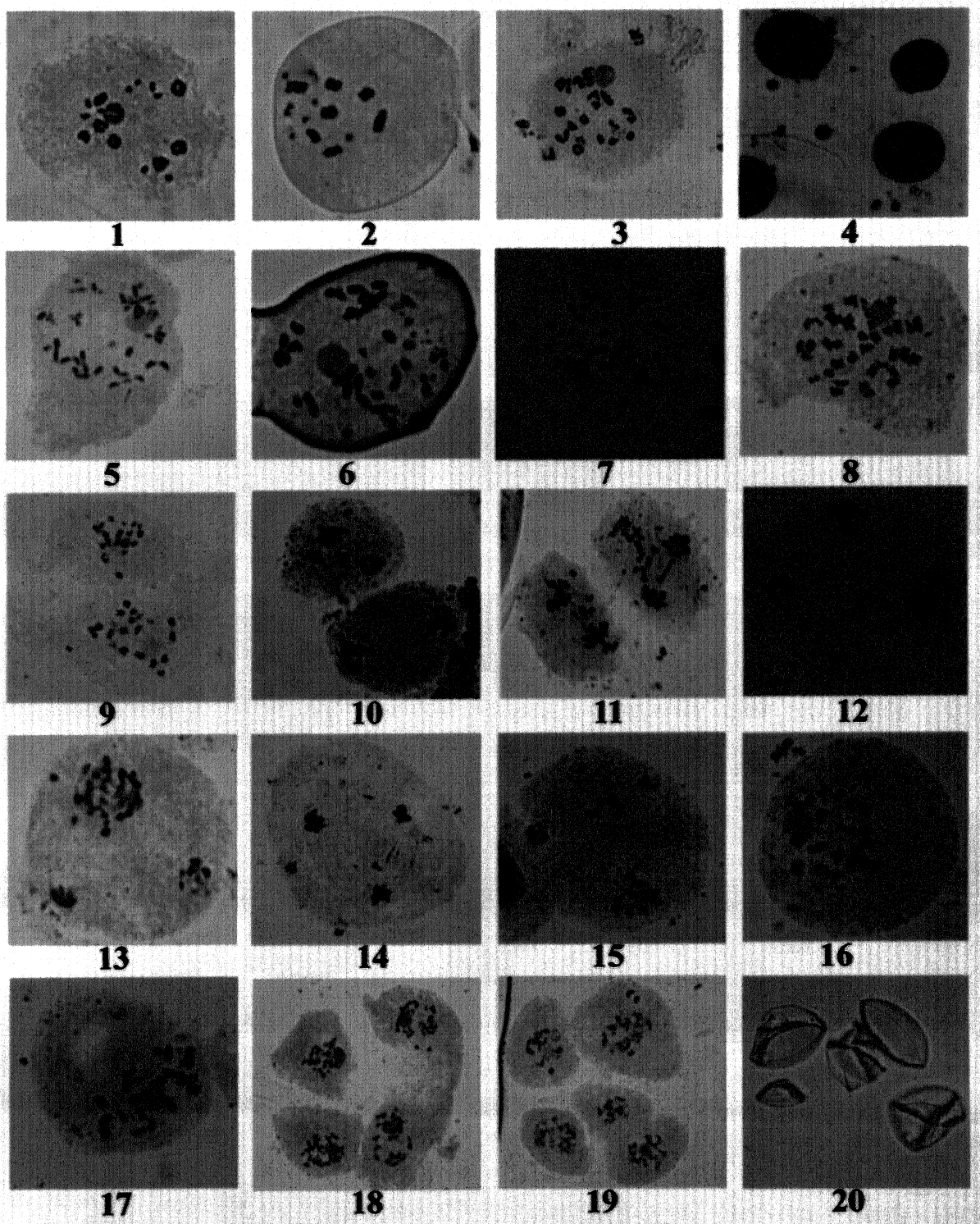


PLATE 18: CYTOLOGICAL OBSERVATIONS IN GOS HYBRID 5

1. BC1 (GO): diakinesis 7IIG + (5_I +2_{II})O.
2. H1 (pearl millet x *P. squamulatum*): diakinesis 21_{II}.
3. GOS hybrid 5: pachytene showing condensation difference in the chromosomes of three genomes.

Aspects of meiocytes that undervent microsporogenesis in mature anthers:

4-5. GOS hybrid 5: diakinesis Note the well-developed exine wall while diakinesis still going on.
6. GOS hybrid 5: diakinesis (9_I +17_{II}).
7-8. GOS hybrid 5: diakinesis.
9. GOS hybrid 5: metaphase.
10. GOS hybrid 5: dyad.
11-12. GOS hybrid 5: late anaphase I.
13-14. GOS hybrid 5: tripolar anaphase I with three nucleoli.
15. GOS hybrid 5: tetrad.
16. GOS hybrid 5: microcytes of different sizes, with different amounts of chromosomes.
17-18. GOS hybrid 5: two and three microcytes with different chromosome numbers attached showing cytomixis had occurred at an early stage.
19. GOS hybrid 5: released microspores with microcytes.
20. GOS hybrid 5: pollen (sterile).

PLATE 18: CYTOLOGICAL OBSERVATIONS IN GOS HYBRID 5

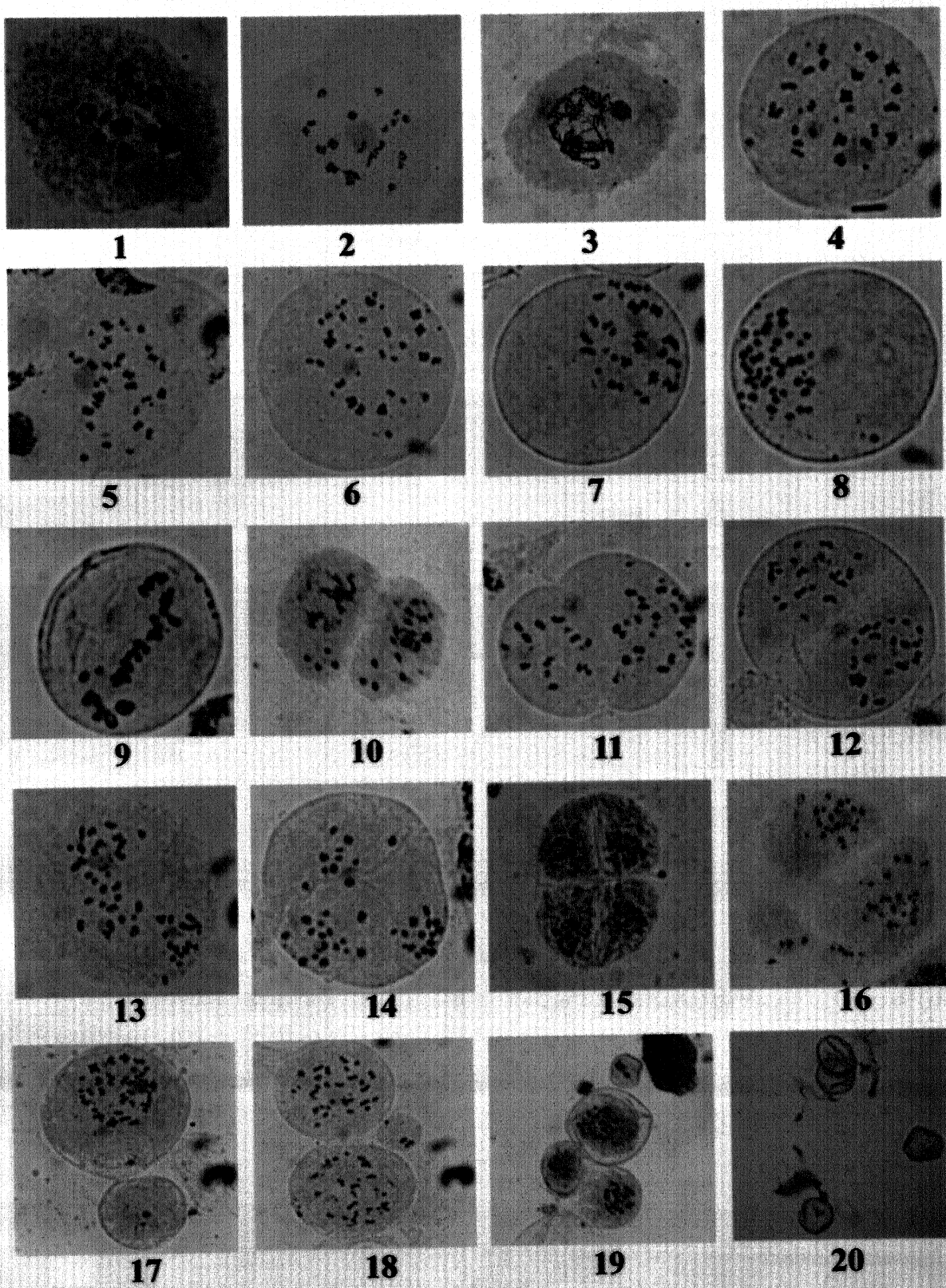


PLATE 19: MEIOSIS IN GOS HYBRID 6 AND HYBRID 7

1. BC_1 (GO): diakinesis $7_{II}G + (5_I + 2_{II})O$.
2. H1 (pearl millet x *P. squamulatum*): diakinesis (21_{II}).
3. GOS hybrid 6: (pachytene showing condensation difference in the chromosomes of three genomes).
4-6. GOS hybrid 6: diakinesis (with higher associations like trivalents and quadrivalents).
7. GOS hybrid 6: metaphase I (9 smaller univalents of *P. orientale*).
8. GOS hybrid 6: anaphase I (with univalents at plate).
9. GOS hybrid 6: telophase I.
10. GOS hybrid 6: pollen (sterile).
11. GOS hybrid 7: diakinesis (with many univalents).
12-13. GOS hybrid 7: metaphase I.
14. GOS hybrid 7: anaphase I.
15. GOS hybrid 7: tripolar anaphase I.
16. GOS hybrid 7: pentad.
17. GOS hybrid 7: hexad.
18. GOS hybrid 7: heptad.
19. GOS hybrid 7: meiocytes (size difference present).
20. GOS hybrid 7: pollen (sterile).

PLATE 19: MEIOSIS IN GOS HYBRID 6 AND HYBRID 7

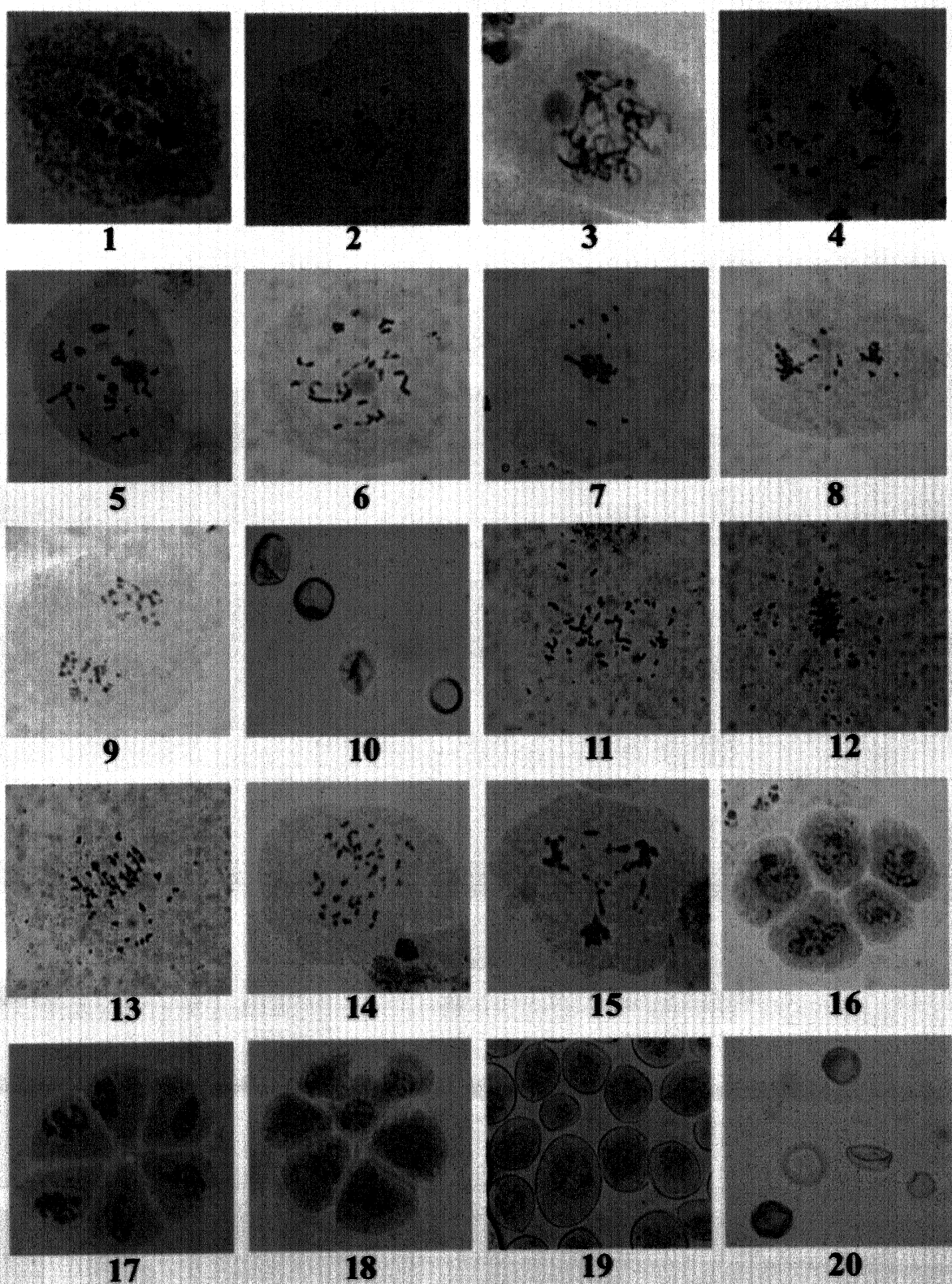
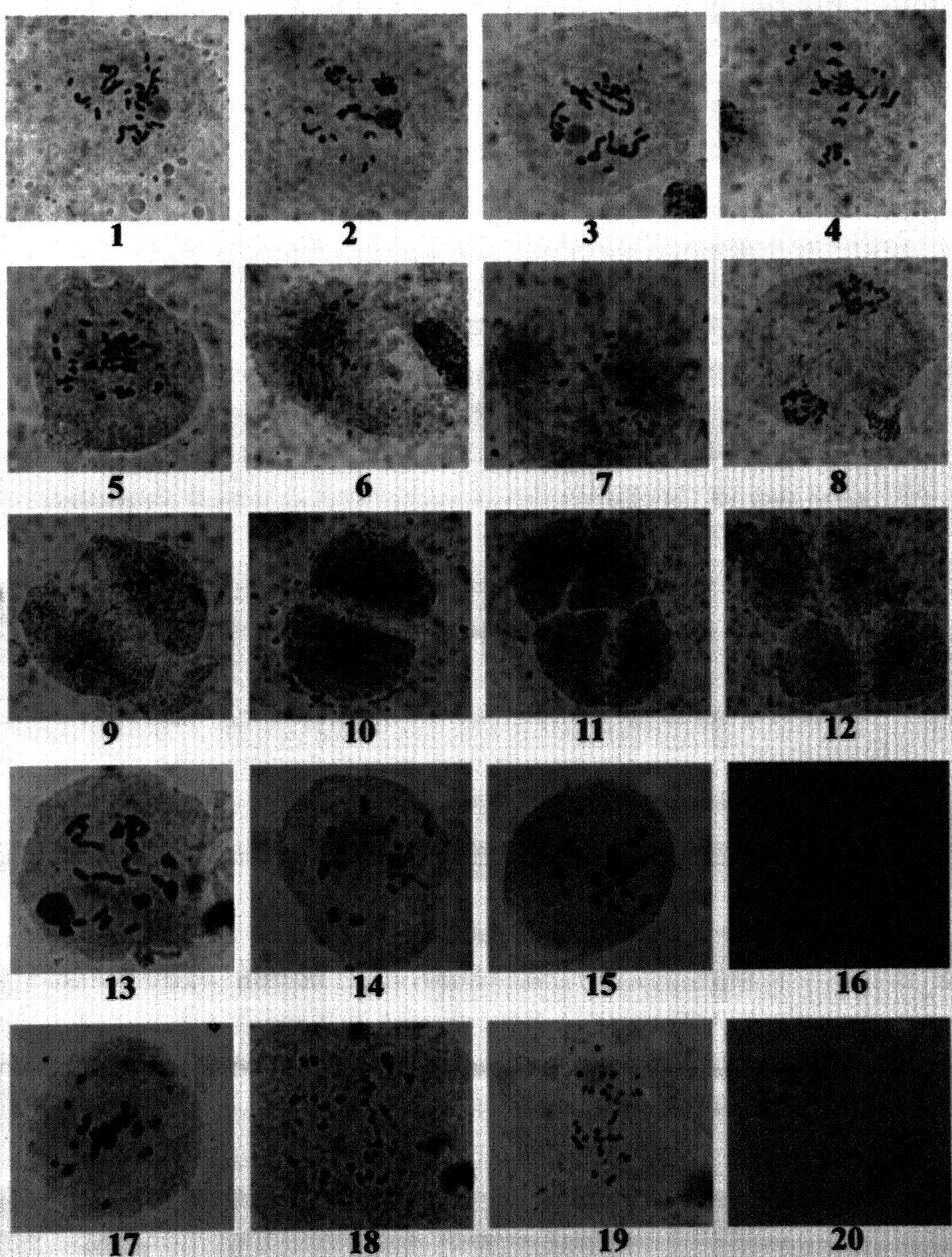


PLATE 20: MEIOSIS IN GOS HYBRID 8 AND HYBRID 10

1-3. Hybrid 8: diakinesis 2n=44 (note the presence of multivalents).
4. Hybrid 8: non-orientation of chromosomes at anaphase I.
5. Hybrid 8: metaphase I with 11 univalents.
6. Hybrid 8: anaphase I.
7. Hybrid 8: metaphase I with 8 univalents.
8. Hybrid 8: telophase I (one small chromosome as laggard probably of *P. orientale*).
9-10. Hybrid 8: dyad.
11-12. Hybrid 8: tetrad.
13. Hybrid 10: diakinesis 2n=35 ($3_I + 16_{II}$).
14. Hybrid 10: diakinesis ($2_I + 15_{II} + 1_{III}$).
15. Hybrid 10: diakinesis ($14_{II} + 1_{III} + 1_{IV}$).
16. Hybrid 10: metaphase I (7 univalents).
17. Hybrid 10: metaphase I (with many univalents).
18-20. Hybrid 10: anaphase I (2n=35).

PLATE 20: MEIOSIS IN GOS HYBRID 8 AND HYBRID 10



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